MacDougal

Reversible variations in volume, pressure, and movements of sap.

REVERSIBLE VARIATIONS IN VOLUME, PRESSURE, AND MOVEMENTS OF SAP IN TREES

BY

D. T. MacDOUGAL

Published by the Carnegie Institution of Washington

Washington, March, 1925

CARNEGIE INSTITUTION OF WASHINGTON
Publication No. 365

PRESS OF GIBSON BROTHERS, INC.
WASHINGTON, D. C.

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INTRODUCTION.

Variations in the thickness of stems which are independent of growth were probably subjected to systematic observation for the first time by Kraus in 1881.[1] Since that time measurements by more accurate methods and with improved appliances have made available a great mass of information on the subject. Previous to the publication of my suggestion as to the part which might be played by tensions on water and air-bubbles in the wood, in 1921, these variations were ascribed to changing turgidity in growing or living cells.[2]

The reversible variations of herbaceous stems or of the thickened members of succulents are due chiefly to varying turgidity in living cells, although, as Bode has shown, the vessels and wood cells of such stems expand and contract with the tension set up in the transpiratory surfaces. The tracts of woody tissue in which the water column under varying tension exists in the trunk of a tree are so thick and show alterations of such amplitude that their behavior is of very great importance in this connection.

Continuous records of the daily alterations of trees are easily made, and there is now an accumulation of such records in which the variations of several trees in all stages of growth and development and under various environmental conditions are available. The use of the dendrograph entails no disturbance of the action of the tree in any manner. The records are capable of analyses in such detail as to yield valuable information on several forms of activity of the tree. Among these the state of the water-column in the wood cells and vessels is plainly discernible. This direct connection has been used as a basis for a restudy of the ascent of sap in trees. Experimental tests have been made by which the transpiration stream has been modified by defoliation and girdling. Some distinct results were obtained in the measurement of exudation or sap pressures in which the apparatus was connected directly with the tracts of tissue in which such pressures originate. Some attention was also given to an attempt to make out the way in which material passes from the leaves toward the root.

[1] Kraus, G. Die tägliche Quellungsperiode der Pflanzen. Abhandl. d. Nat. Gesell., Halle, 15, 1881.

[2] MacDougal, D. T. Growth in trees. Carnegie Inst. Wash. Pub. No. 307. 1921.

The student of growth in trunks of trees is primarily concerned with the activity of terminal cones of embryonic cells and of the paper-thin layer of cambium which sheathes the woody cylinder. Measurements of changes in diameter or circumference necessarily include the variations in sectors of the entire trunk. In the use of the dendrograph the bark of pine trees is sliced away to a thinness of less than 1 mm. to obtain suitable contacts or bearings, so that errors from changes in such dead cells are reduced to a minimum. It is not possible to measure the variation in the cambium or phloem of a trunk without including some of the central cylinder of wood. The nature of the changes which the wood cells undergo, however, are of a different kind from those of the living cells, and it is shown in the pages that follow how the dendrographic record may be analyzed and the temporary fluctuating or reversible variations in the wood may be separated from those characteristic of living cells. This differentiation rests upon the fact that while living cells undergo changes by distention from osmotic action and shrinkage from loss or turgidity, the wood cells, once they have obtained full size and become conduits for upwardly moving transpiration streams, undergo changes in volume only in response to variations in the cohesion tension of the water-column which extends from the roots to the leaves in living trees. In actual practice, variations in diameter may be safely assigned to either cause upon the character of the dendrographic record. The variations in question are of a magnitude by which they may amount to as much as 1 part in 1,250 of the diameter at the base of the trunk of a Monterey pine tree, where it has a thickness of 40 to 50 cm., 1 part in 700 midway of the trunk, and as much as 1 part in 170 in the stems of young trees with a diameter of 10 cm. Presumably the terminals of active large trees behave in a similar manner.

Reversible variation would seem to be greatest in living-cell masses and in recently formed wood-cells. This conclusion is supported by dendrographic measurements of trunks of Monterey pine, cutting away the wood of the two previous years and making the bearings on the earlier wood. The reversible variation of this internal woody cylinder varied from 1 part in 2,000 to 1 part in 8,000. This arrangement entailed the removal of living cell-masses and of recently formed wood, but not all of the tracts through which ascending sap streams may flow, as it has been found by observations described in a subsequent section of this paper that the complex column of water under tension in which the liquid moves upward in the stem fills the second, third, and fourth layers of the wood of the Monterey pine. The fifth layer is sometimes included in the system, and the liquid in the outermost or first layer is continuous with it.

NATURE AND SEAT OF REVERSIBLE VARIATIONS.

My first attempt at an interpretation of the nature of reversible variations in woody cells was published in 1921 and was based upon the double assumption that gas-bubbles were present in the conducting tracts and that the membranes of the bordered pits which offer a passage from one tracheid to another were intact and hence would hold a bubble in the lumen in which it originated.[1]

It has recently been made clear that the membranes of the bordered pits are perforated, and that bubbles, if present, could be forced through them by an osmotic pull of less than 3 atmospheres, according to Professor I. W. Bailey. It has also been shown by various workers that the tracts of wood followed by upwardly moving currents include very few gas-bubbles. My earlier conclusion that the excessive withdrawal of water from the wood-cells would be followed by a shrinkage due to the increased tension of the remaining contents remains essentially correct.

The water deficit which would increase from the base of the trunk toward its summit would be accompanied by an increased cohesion tension, according to this conception. Relief or lessening of the water deficit by checked transpiration or by an additional supply from the base would be followed by diminished cohesion tension, by a consequent return of the tracheary elements to their normal dimensions, and a consequent swelling of the stem. Direct observations in confirmation of this requirement have been made by Bode.[2] Contraction followed increased transpiration in single vessels, and entire stems of small woody plants were seen to contract when transpiration was increased suddenly. The use of a pump to introduce variations in the pull on the shoot was not seen to make perceptible changes in dimensions of stems. The osmotic pull which may be exerted by the transpiring cells of the leaf generally amounts to many atmospheres, and no marked results might be expected from changes of less than 1 atmosphere in the tension, although, as I have shown elsewhere, the use of a pump in this manner may be seen to accelerate the flow of dye solutions through stems. Bode also found that the removal of the leaves was followed by an increase in diameter of small stems. A similar immediate effect has been seen following defoliation, partial or complete, in the Monterey pine. The facts given in a later section of this paper establish beyond all doubt that the daily reversible variations in the trunks of trees are intimately linked with the course of transpiration and with the daily stomatal program. Contraction or shrinkage is an invariable attendant of lessened water-balance due to an excess of transpiration over water supplied, whether the contrac-

[1] MacDougal, D. T. Growth in trees. Carnegie Inst. Wash. Pub. No. 307. 1921.

[2] Bode, H. B. Beiträge zur Dynamik der Wasserbewegung in den Gefäss-Pflanzen. Jahrb. f. Wiss. Botan. 62, 92–127. 1923.

tion be due to increased cohesion tension or reduced turgidity. Since it is possible to distinguish the action of living cells from that of woody cells, it is found that the changes in volume of stems and trunks of trees may be made to yield decisive evidence as to the nature of the mechanism of the ascent of sap and the translocation of material.

The dendrographic records which have formed the basis of my extended studies in growth now include continuous and complete records of changes in trunks of living trees for a total of more than 100 years. More than half of this total is represented by measurements of the Monterey pine, which is native and abundant about the Coastal Laboratory, grows rapidly, and, having a long season, lays down as much as 7 or 8 mm. of wood in a single year, extending its terminals a few centimeters or as much as 2 meters during this period. When it is added that the living material is so accessible that about 200 individuals have been under instrumental observation or experimental tests it may be seen that the work has been conducted under advantageous conditions.

SCHEME OF STRUCTURE OF TREE TRUNKS.

An active tree may be visualized as a tapering, many-layered cylinder of wood sheathed with cortex and bark. Immediately external to the wood is the single-layered cambium of embryonic cells with a thin wall and comparatively dense protoplasm. During the growing season, externally and internally to the cambium, the derivatives form a heavy layer of dividing cells rapidly passing into the elements of the xylem on the one hand and into the cortex on the other. The passage of material in solution through this membrane in its expanded condition would be a serious matter. Thus, if it were proven, which it is not, that sugars descend the stem in sieve-tubes and elongated elements of the phloem, the passage of such material through this layer in a condition of active growth would present some serious physical difficulties.

The wood cells formed internally to the cambium pass rapidly into the mature stage, so that in all likelihood it is only the 3 or 4 or at most 15 or 20 outermost wood cells which retain any of the plasmatic material. The tracheids therefore soon become cleared and offer conditions of conduction radially in the ray tracheids and vertically in the main part of the layer. The newly formed wood, however, is not connected directly with the xylem of the leaves, and there is therefore no direct transpirational pull on the liquid in the wood cells of the current year in the Monterey pine. As will be discussed later, the liquid in these cells is in direct lateral connection with the main water column terminating in the leaves and which is in a state of cohesion tension.

Sheets of parenchymatous cells with intact walls extend radially through the wood, being separated from the cavities of the tracheids by the characteristic thin walls of such elements. Solutions may pass between these turgid living elements and the wood cells by osmotic action; liquid may be forced into the wood by "exudation pressure" or taken from the wood into the parenchymatous cells by endosmosis, according to the relative state of turgidity of the cells.

The wood-cells open into each other vertically, or parallel to the axis of the trunk, and tangentially, but not radially. The wood cells of one layer therefore connect by direct openings with each other through the walls radially placed, so that a liquid in a layer may escape from it only by the ray tracheids, which run radially and offer much the same communication by bordered pits as the vertical tracheids.

The possibility of the presence of perforations in the membranes of the bordered pits is of great importance in the matter of the transportation of material in the ascending sap stream. The examinations of the wood cells were not adequate to determine this point, but their presence in other conifers makes it reasonable to assume their existence in the Monterey pine. These perforations are from 3 to 5μ across in the wood of the larch, according to Professor I. W. Bailey. The reversible daily variations are due to changes in this shell of 1 to 4 seasonally formed layers of wood and the living cells external to the wood. If the change in this hollow cylinder could be calculated directly on its thickness, it might amount to as much as 2 or 3 per cent; which obviously would be a feature to be taken into account in measuring the flow of material through the included layers.

With reference to external conditions, the Monterey pine and other coniferous trees may be regarded as hollow cylinders of woody cells infiltrated with a continuous water column connected externally with the phloem by the thin-walled ray cells. At the upper terminus the wood cells come into connection with the living cells of the leaf. The woody cylinder at the base runs out into thousands of thin rootlets in which the xylem is incased by the living, rigid endodermis and by the living cortex bearing the root hairs.

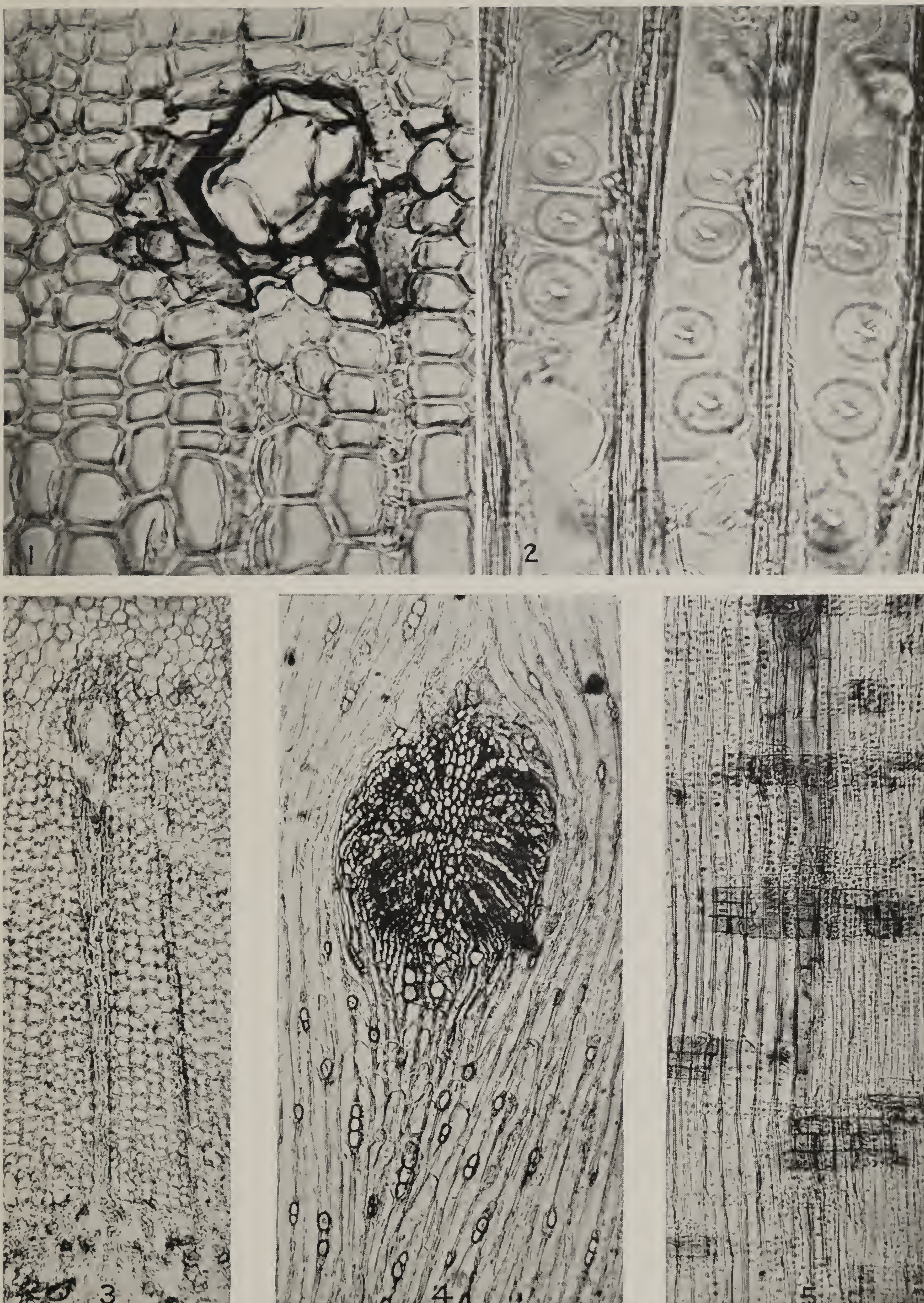
The whole mechanism is one in which water is lost from the free surfaces of the living cells at the apices or upper terminals of the wood, the living cells pulling water from the wood cells by osmotic action. The water in the wood cells being in a continuous "column" or network, the withdrawal of water from the system sets up a tension quickly transmissible to all parts of the system, as tested by the consequent contraction of the wood.

General inquiries during the last three decades have brought out a vast amount of information concerning root-pressure, ascent of sap, and translocation of material in woody stems. Of the various explanations of the mechanism of such movements the theory of Dixon as to

the presence of a continuous column of water in trunks extending from the roots to the leaves has withstood all serious criticism and furnishes the essential conditions by which water might be pulled up the length of any stem by evaporation from the walls of cells into the intercellular spaces in the leaves. These turgid cells replace their losses by endosmosis from the xylem with which they are in contact, and in which a complex column of water extends to the source of supply in the soil.

Many features of this action have been obscure and the whole matter of the translocation of carbohydrates and proteins from the leaves through the stems has been in doubt. Solution of these problems has seemed to be most readily attainable by intensive study of a single species. Such a study may be most profitably made when the actual structure of the tree is taken into account. Not much reliance has therefore been placed on generalizations as to the action of conifers, but all considerations have been referred to the species of pine to which so much attention has been given. Measurements of its growth and reversible variations for extended periods are now available. Mr. L. H. Daugherty, of Stanford University, has made a preliminary study of the anatomical features of the wood which seem to be of most importance in changes in volume and transport of material, and the following section is devoted to the facts which he describes.

PLATE 1

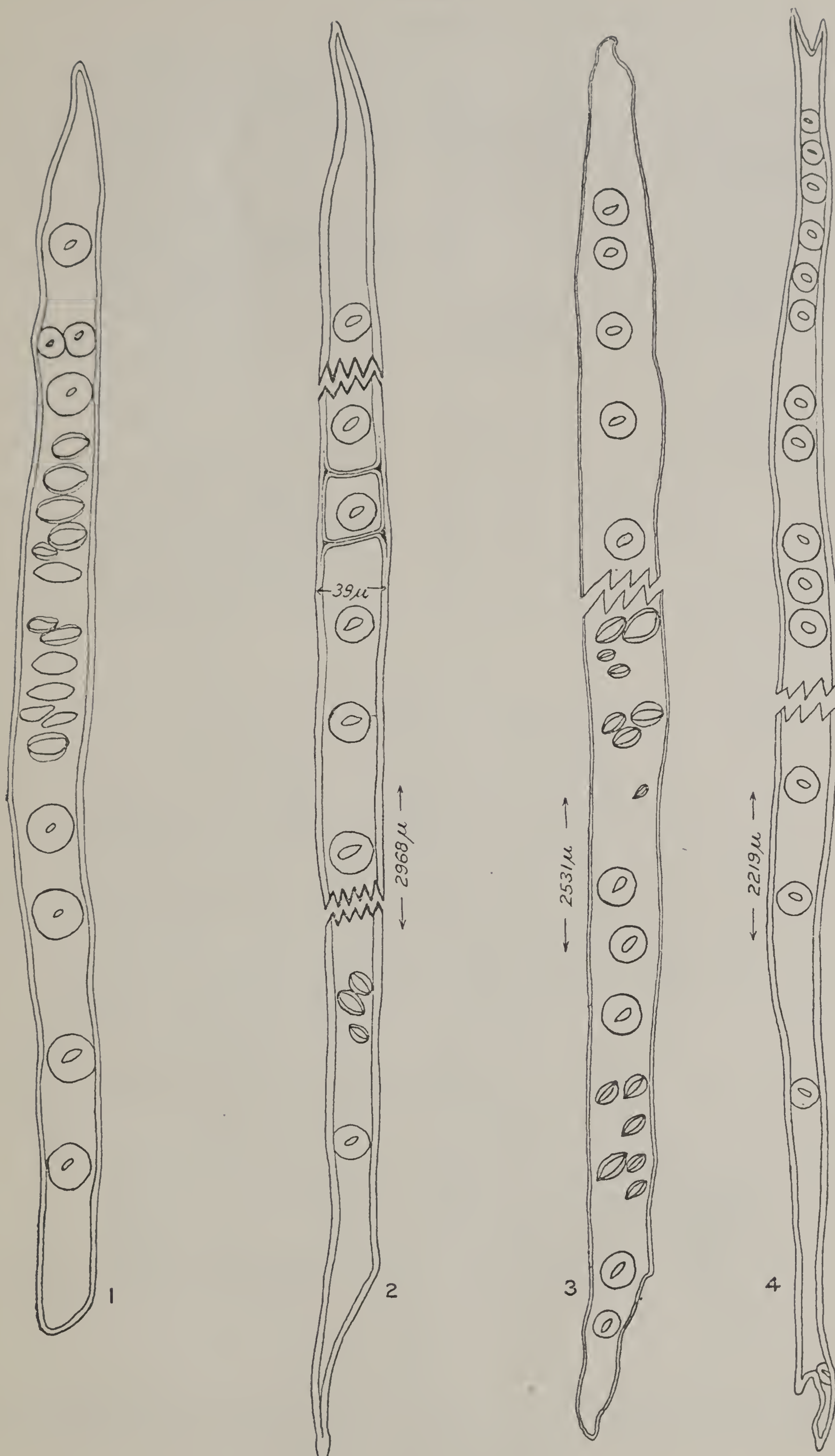


PINUS RADIATA.

(1) Transverse section; resin duct passed by medullary ray. Note the modified medullary ray tracheid with the spiral band at side of resin duct. (2) Radial section; bordered pits and bars of *Sanio*. (3) Transverse section; young stem showing fusiform ray originating in primary resin duct. (4) Tangential section; leaf-trace in young stem. (5) Radial section; dark cells running in a longitudinal direction are the end of a resin duct.

After photomicrographs by L. H. Daugherty.

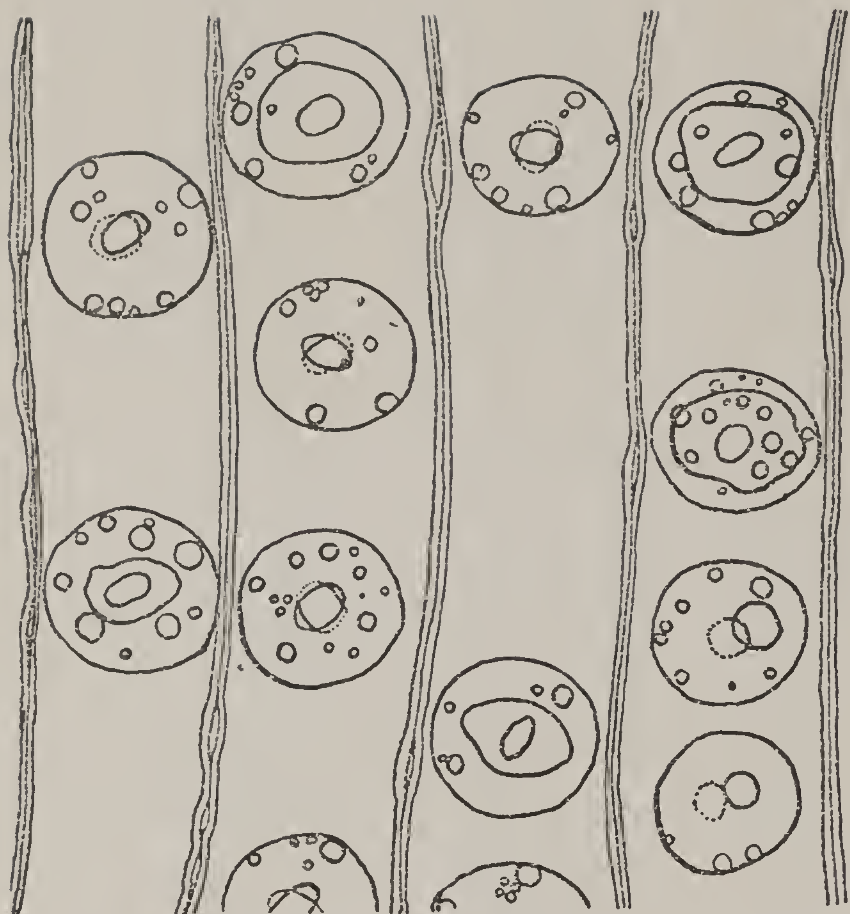
PLATE 2



TRACHEIDS.

(1) Tracheid of primary xylem. (2) Tracheid containing radial cross-bars. (3) Tracheid of spring wood. (4) Tracheid with V-shaped ends.

After drawings by L. H. Daugherty.



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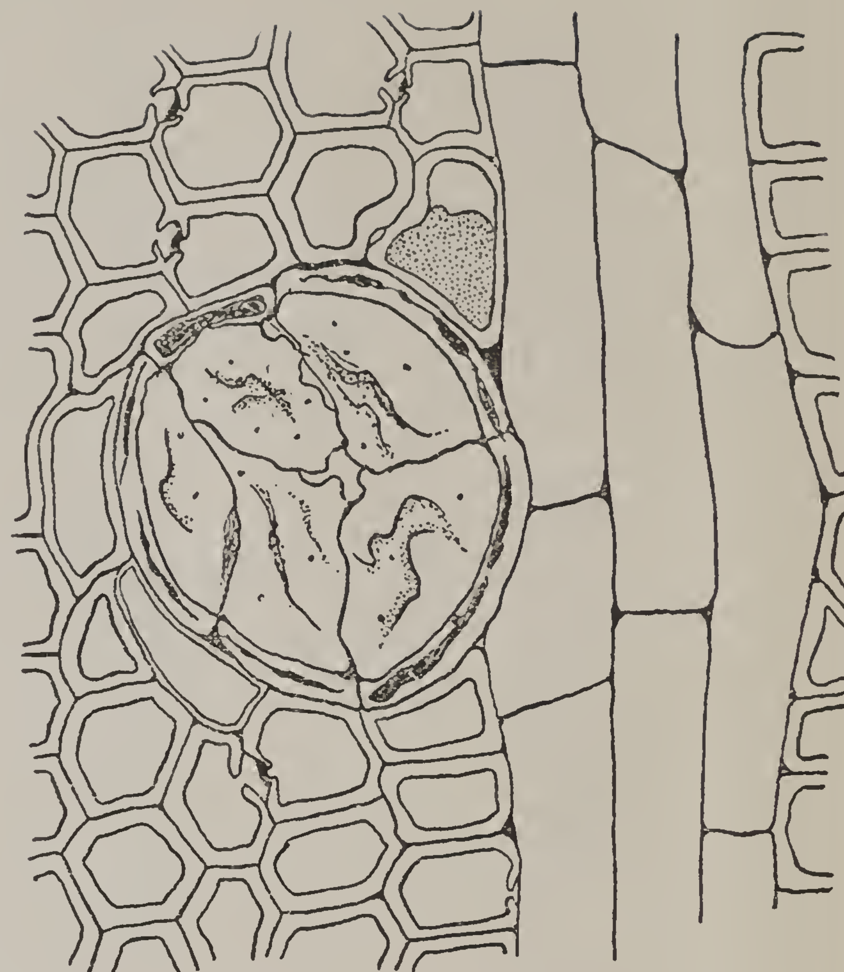

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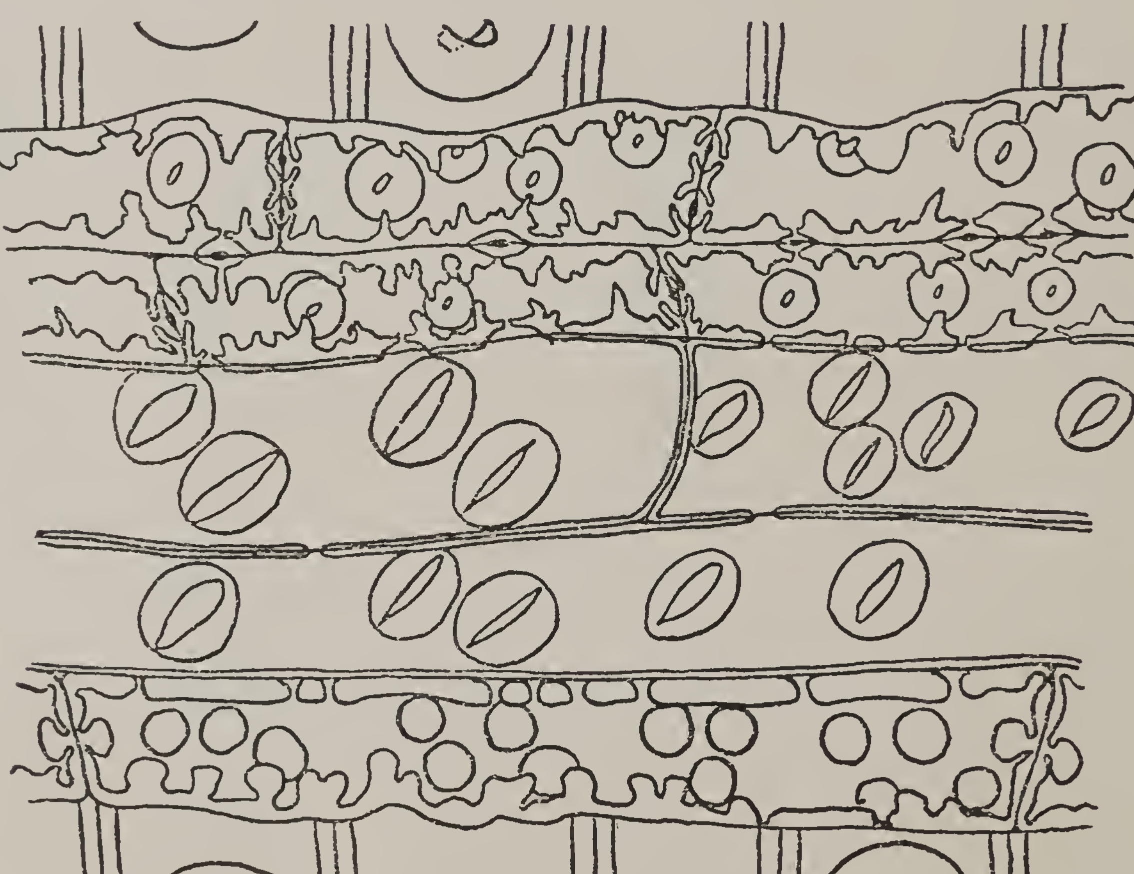

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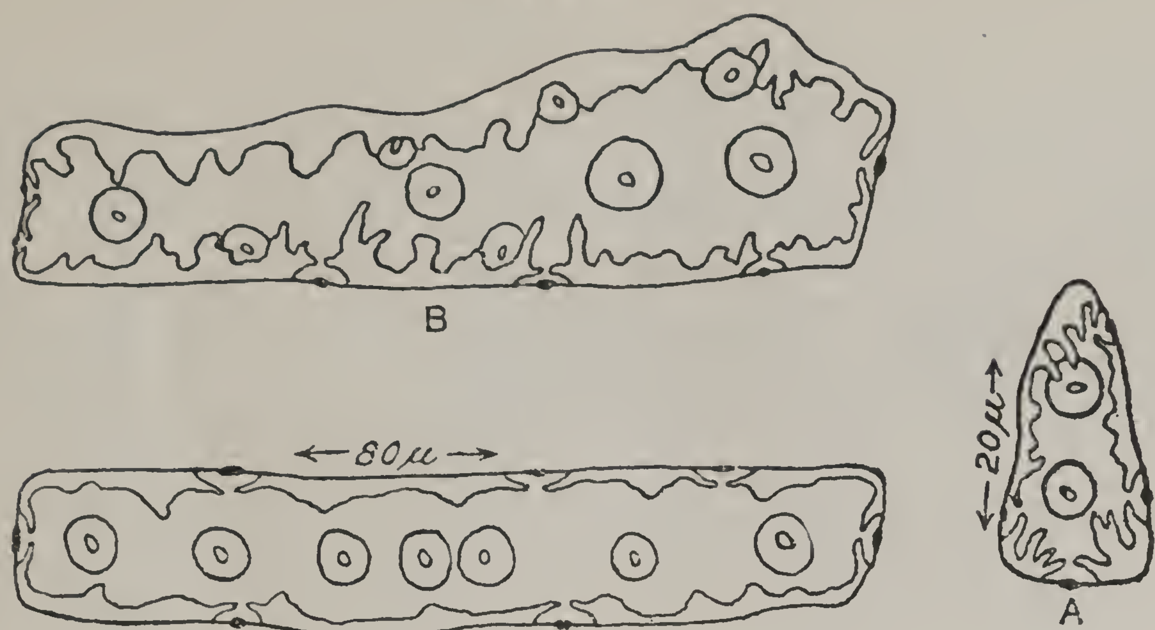
CELLS OF RESIN DUCTS.

(5) Radial section; pits with deformed borders. (6) Transverse section; resin duct plugged by tylosis, and passed by fusiform medullary ray. (7) Radial section; showing elements of medullary ray.

After drawings by L. H. Daugherty

PLATE 4



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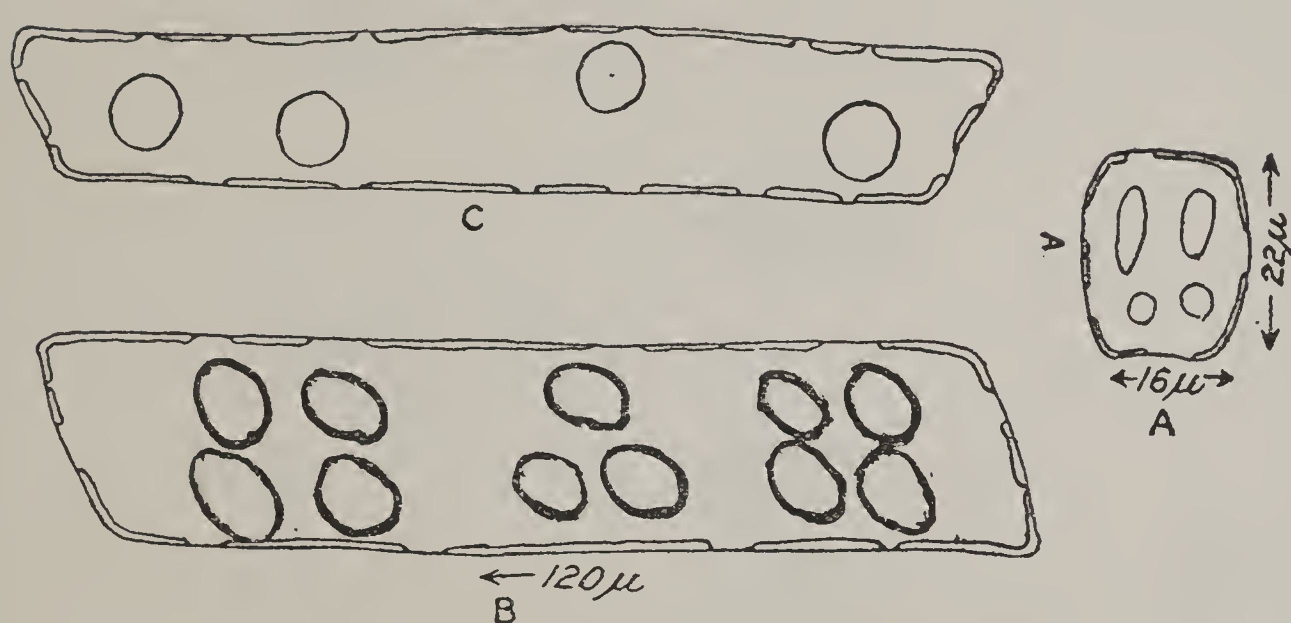

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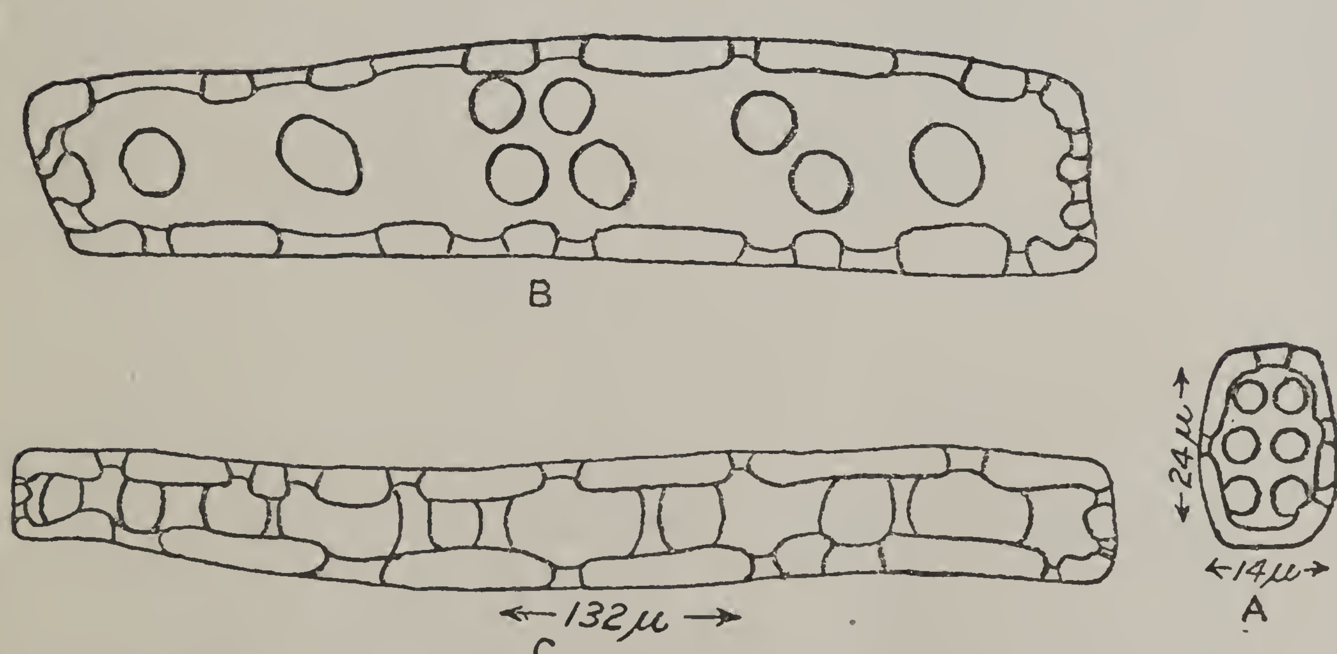

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MEDULLARY RAY CELLS.

(8) Tracheid of medullary ray: *A*, tangential view; *B*, radial view; *C*, transverse view. (9) Thin-walled parenchyma cell of medullary ray: *A*, tangential view; *B*, radial view; *C*, transverse view. (10) Heavy-walled parenchyma cell of medullary ray: *A*, tangential view; *B*, radial view; *C*, transverse view.

After drawings by L. H. Daugherty.

PLATE 5

11

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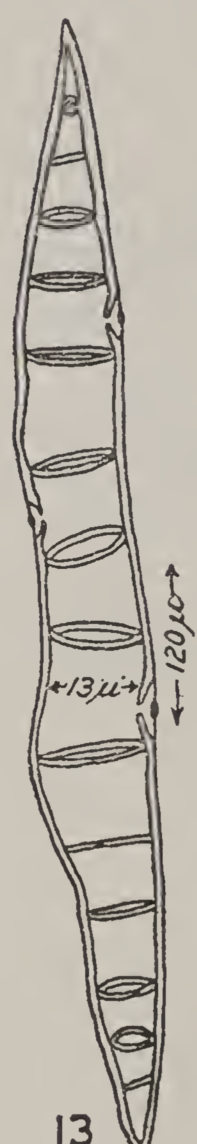

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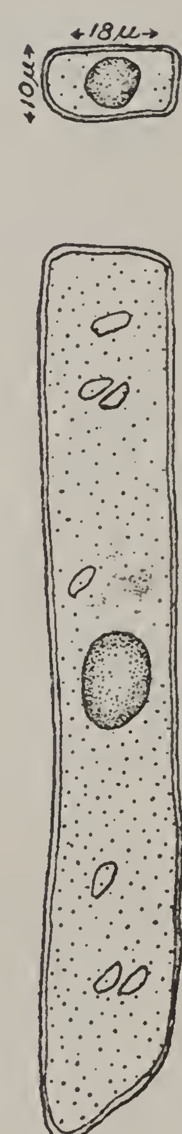

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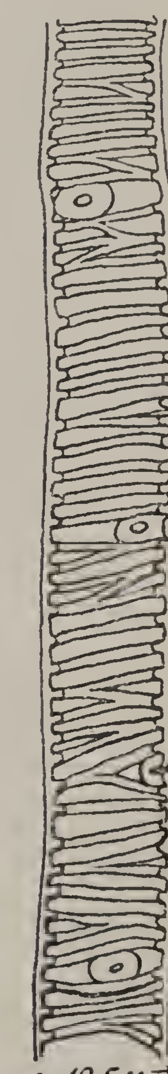

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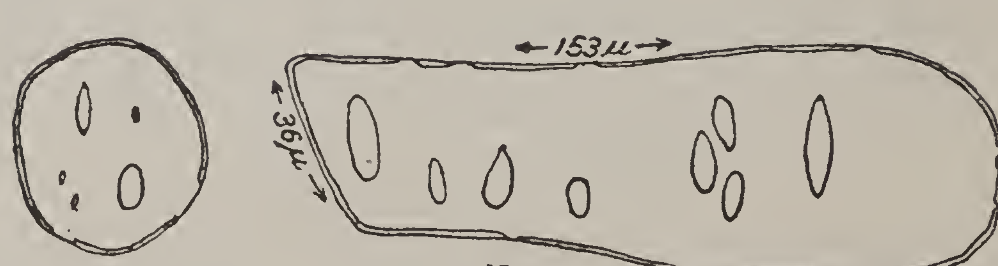

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CELLS OF RESIN DUCTS.

(11) Radial section; medullary-ray cells, starch-containing cells. (12) Transverse section; tracheids in heart-wood containing crystal. (13) Annular tracheid. (14) Resin cell. (15) Parenchyma cell of resin duct. (16) Spiral duct. (17) Pith cell.

After drawings by L. H. Daugherty.

ANATOMICAL DESCRIPTION OF THE XYLEM OF PINUS RADIATA.

By L. H. Daugherty.

The detailed results of my examination of the xylem of *Pinus radiata* are given in a form modified from that used by Moll and Janssonius.[1] In the description of the elements of the stem, *R* designates radial measurements, *T* tangential, and *L* longitudinal dimensions. The Greek letter μ, as is customary, is used as the symbol of the micron, or 0.001 mm. Large numbers were measured when possible, but in the case of the annular tracheids but two were seen. I have to thank Dr. L. B. Becking and Professor G. J. Peirce for assistance in the revision of the manuscript.

MACROSCOPIC CHARACTERS.

The wood is light, rather soft, not strong, brittle, and as a rule close-grained and compact. The duramen, heartwood, is but little darker than the alburnum, sapwood. The heartwood can not be distinguished from the sapwood in most of the young trees, but may be seen in old trees. Penhallow [2] gives the following physical properties for *P. radiata:*

Specific gravity	0.4574
Percentage of ash residue	0.30
Approximate relative fuel value	45.60
Coefficient of elasticity in kilograms on millimeters	979.00
Ultimate transverse strength, in kilograms	316.00
Ultimate resistance of longitudinal crushing, in kilograms	6,680.00
Resistance to indentation to 1.27 mm., in kilograms	1,687.00

The growth rings, or so-called annual rings, range in thickness from 19 to less than 2 mm. There is a large amount of irregularity in the thickness of the different annual rings, and, in addition, there may be a difference in the thickness of the same annual ring at different levels in the stem. The stems examined formed the largest rings in the first 13 years, while the rings formed after the thirteenth year showed a marked decrease in width. As a rule, there is a gradual transition from spring wood to summer wood, and when two rings are formed in the same year there may be a gradual transition from the summer wood to the spring wood. In large rings there is much more spring wood than summer wood. For example, in rings 18 mm. in thickness the summer wood measures approximately 3 mm. When more than one ring is formed in a growing-season, the summer wood is not more than 1 mm. and in many cases not more than 0.5 mm. in thickness.

MICROSCOPIC CHARACTERS.

Topography.

The bulk of the stele, the *Grundmasse*, consists of ordinary tracheids with main axis running in longitudinal direction. Scattered among the ordinary tracheids, and having their main axis in the same direction, one may occasionally find small annular tracheids. These tracheids are very rare and possibly are all situated in or near the primary xylem. The ordinary tracheids appear to have the same general structure throughout the stele, with the exception of the first tracheids formed in the primary xylem. These tracheids differ in having larger medullary-ray pits than the tracheids in the secondary xylem.

[1] Moll, J. W., and H. H. Janssonius. Botanical pen-portraits. The Hague. 1923. Mikrographie des Holzes (vol. 1, 1906; vol. 2, 1908; vol. 3, 1914).

[2] Penhallow, D. P. North American Gymnosperms. Boston, 1907.

The large medullary-ray pits are found in most cases with very narrow borders, but may be simple.

The primary medullary rays run through the stele in a radial direction from the pith to the cortex, and from 13 to 20 may be counted in an ordinary transverse section. The secondary medullary rays are numerous, many of them originating in the primary xylem. In a transverse section of a one-year-old stem 42 secondary medullary rays were found and only 18 primary medullary rays could be counted. In transverse sections of older stems the medullary rays are separated by from 2 to 12 tracheids, the average being 8. By counting the medullary rays in a tangential section I found that there was an average of 52 medullary rays to a section 1 mm. square. The rays are of three types: simple, fusiform without resin ducts, and fusiform with resin ducts. The fusiform rays are not as numerous in *Pinus radiata* as they are in many other gymnosperms. I found the simple medullary rays to be 1 cell wide and from 1 to 15 cells in height, the average being 4 to 5 cells high. The fusiform rays are but 1 cell wide at the upper and lower extremities, and from 2 to 4 cells in width in the center. As a rule the resin ducts in the rays are near the center, but they may be near the upper or lower part of the ray.

The rays consist of three types of cells: the thin-walled parenchyma cells, the thick-walled parenchyma cells, and the tracheids. It might be added that the thin-walled parenchyma cells are of two types: the ordinary medullary-ray parenchyma cell and the parenchyma cells lining the resin ducts, which are ordinarily called resin cells. The tracheids and the thick-walled parenchyma cells commonly appear at the upper and lower edge of the medullary rays. Cases were found, however, where the tracheids were in the middle of the rays, and rarely one can find medullary rays containing no parenchyma cells. In a tangential section 0.25 mm. square I counted 85 thin-walled parenchyma cells and 17 heavy-walled cells. If this count can be used as a criterion, the thin-walled parenchyma cells must make up 85 per cent of the medullary rays. All the cells of the medullary rays have their greatest axis running in a radial direction, and as a rule are taller than they are wide. In some gymnosperms, e. g., *Ephedra viridis*, I found medullary-ray cells with their greatest axis running in a longitudinal direction, but I could find no cases of this in the stele of the Monterey pine.

All resin ducts not in fusiform rays run in a longitudinal direction. They are formed schizogenously, and consist of two kinds of cells. The duct is surrounded by ordinary resin cells which in most cases are surrounded by a layer of parenchyma cells that closely resemble the resin cells. The outer layer of parenchyma cells are longer than the resin cells, and have a more transparent protoplast. The resin ducts are rather numerous in the outer face of the summer wood. They may be found, however, scattered throughout the growth-ring. From 1 to 2 resin ducts appear in each primary vascular bundle, in most cases 2. The resin ducts are from 7 to 14 cm. in length and end blindly. They run parallel to the inner portion of the annual ring, and due to the stresses placed upon the stem may form steep spirals. This tendency to ascend the stem in a steep spiral makes it difficult for one to trace the course of the resin ducts in stems where this occurs.

The spiral ducts are restricted to the protoxylem, and are found next to the pith, or medulla. I counted the spiral ducts in one stem and found 440. In a stem of *Pseudotsuga taxifolia* I counted 360, but I do not know how much the number may vary in different stems of the same species.

The pith, or medulla, in the center of the stem measures from 1 to 3 mm. in diameter. It consists of thin-walled parenchyma cells with their greatest axis running in a longitudinal direction. Near the protoxylem the cells are

longer, smaller in diameter, heavier walled, and the walls are slightly lignified. The pith remains continuous in the stem, and does not break up into disks as it does in certain other gymnosperms. To make this more clear, I might add that if one examines the pith in the stem of *Pinus coulteri* it can be easily seen that the pith does not form a continuous column, but is broken up by air-spaces. (Plate 1.)

DESCRIPTION OF THE ELEMENTS

I. Tracheids.

a. Ordinary Tracheids (number measured, 1,400).

R, 13 to 68μ; *T*, 13 to 62μ; *L*, 628 to 4,150μ. Tracheids 4 to 8 sided; ends ordinarily pointed, but may be blunt. In some cases the ends are V-shaped, due to the tracheid being stopped during sliding growth. Walls in cells of spring wood 2 to 3μ; walls of cells in summer wood 4 to 7μ. Middle lamella thin, stains blue in analin blue and does not take safranin. Secondary layer rather thick in summer wood and forming bulk of cell-wall. Tertiary layer very thin and can be seen only in summer wood. Stratification of cell-wall not visible without the aid of reagents. Striations can be seen on cell-wall with aid of Schultze's solution. The striations are in spirals, and may run clockwise or counter-clockwise. Tracheids are sometimes found with strengthening bars running through the lumen in a radial direction. These bars are the "Querbalken" of Strasburger and are described on page 32 of his Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen.[1] Bailey has described septate tracheids in certain pines, and it might be possible that some of the tracheids of this pine were septate. I do not believe this to be the case, but know no way in which one could tell the difference between strengthening bars and septa when they appear in a radial section. Bars of Sanio appear upon the cell-walls, but they are not common. In some cases I cut a large number of radial sections without finding the bars of *Sanio*, and in other cases they were found in large numbers. These bars are rather narrow and are found on the cell-walls between bordered pits that are rather close together. The pits of the tracheids are all bordered or half-bordered. The outline of the bordered pit is either circular or eliptical on account of the fact that they are slightly flattened at the top and bottom. The measurements of the diameter of the pits are: *R*, 17 to 28μ; *L*, 15 to 24μ. The openings are circular or oblong. In all cases the oblong openings run parallel to the striations of the cell-wall. The borders are large, rather thin, and in some cases not fully developed. The torus of the pit is large, stains deep blue in anilin blue, and its diameter measures 7μ. The bordered pits are usually in single rows, but occasionally occur in pairs. Pits in pairs are rare, being most numerous in primary xylem. The outline of the half-bordered pits elliptical to oblong, in some cases circular. The diameter of the half-bordered pits are: *R*, 12μ; *L*, 9μ. Largest diameter is parallel to striations on cell wall. Openings on bordered side narrowly elliptical; longest axis extending to edge of border. Half-bordered pits are in one or two rows, or they may be irregularly placed. The borders are very narrow, and cases may be found where the pits appear to be simple. (Plate 2.)

With the exception of tracheids near a rapidly dividing cambium, no cell-contents can be found. Crystals are occasionally found in the heartwood. In the heartwood and in wound areas the tracheids may be filled with resin.

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[1] Strasburger, E. Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. (Gustav Fischer, Jena, 1891.)

b. Annular Tracheids (number measured, 2).

R, 12μ; *T*, 13μ; *L*, 120μ. Shape of cell the same as ordinary tracheids, with cell-wall 3μ in thickness. Differing from other tracheids in having ringed thickenings on cell-wall. The bordered pits are circular and measure 9μ in diameter. Bailey [1] found spiral tracheids in certain species of pine, but I find no reference to annular tracheids in any of the literature that I have had the good fortune to read. (Plate 5, 13.)

II. Medullary-Ray Cells.

a. Thin-walled Parenchyma Cells (number measured, 50).

R, 45 to 233μ, average 130μ; *L*, 16 to 36μ, average 24μ; *T*, 13 to 33μ, average 19μ. Cells are 3 or 4 sided prisms. Walls thin, less than 1μ. Walls composed of cellulose not impregnated by lignin. No stratification, striations, or thickenings present. Pits simple or half-bordered. 1 to 4 pits in crossing-field. (By "crossing-field" is meant the section of the radial wall crossed by one tracheid. Burgerstein used this term in his description of gymnosperms.) Pits on radial walls circular or slightly oval, measuring from 9 to 12μ in diameter. Pits on upper and lower walls circular, measuring 8 to 12μ in diameter. Pits on terminal walls either circular or elongated, measuring 2 to 4μ when circular. (It is rather difficult to see the pits on the end-walls, and only a small number were measured.) Half-bordered pits described in I, *a*, and II, *c*. Protoplast contained disk-shaped leucoplasts or amyloplasts. These plastids are thickened at edge and contain starch. They measure 13μ in diameter and 3μ in thickness. (Plate 3.)

b. Heavy-walled Parenchyma Cells (number measured, 20).

R, 71 to 231μ, average 173μ; *T*, 9 to 19μ, average 13μ; *L*, 9 to 21μ, average 14μ. Shape same as that of thin-walled parenchyma cells. Walls thick, 6 to 9μ. Middle lamella thin, but containing local thickenings. Main part of wall consisting of secondary thickening layer. Tertiary layer, if present, can not be distinguished from secondary thickening layer. Walls stain yellow in chlor-zinc-iodide, and take safranin readily. In color and texture the walls resemble the walls of the tracheids, and presumably are composed of the same material. Pits are all simple or half-bordered. Simple pits on radial walls circular, or nearly so, measuring from 6 to 9μ in diameter. Simple pits upon upper and lower walls very large, and so close together that the space between them resembles bars. Pits on terminal walls are small, circular, and from 2 to 6 in number. They measure from 4 to 6μ in diameter. For description of half-bordered pits see Tracheids, I, *a*. Occasionally the thick-walled parenchyma cells are filled with resin in the duramen, or in wound areas; no starch or other materials found in them.

c. Tracheids (number measured, 50).

R, 144 to 236μ, average 178μ; *T*, 6 to 16μ, average 11μ; *L*, 10 to 26μ, average 16μ. Cells 3 or 4 sided prisms, or irregular. Walls thick, with inner surface dentate, or walls thin and having spiral bands. Thin-walled tracheids containing spiral bands are found in cases where tracheid is in contact with resin duct. The walls of all medullary-ray tracheids are lignified, and appear to be the same as the ordinary tracheids in composition, color, etc. Pits all bordered or half bordered. The halo of pit is circular and is 12μ in diameter. Aperture of pit small, the canals or openings circular or oblong. Pits on terminal

[1] Bailey, I. W. Structure of the wood in the pineæ. Bot. Gaz., Chicago 48, 47-55, 1909.

walls similar to pits on radial walls. The terminal walls contain from 1 to 3 pits. As a rule, pits on radial walls are in one row, averaging from 1 to 3 in each "crossing-field." Pits on upper and lower walls same in number and arrangement as those on radial walls. With but few exceptions the cells appear to be empty. They are sometimes found filled with resin in the heartwood or in wound areas.

III. Cells of Resin Ducts.

a. Resin Cells (number measured, 50).

R, 9 to 40μ, average 25μ; *T*, 9 to 40μ, average 25μ; *L*, 45 to 115μ, average 98μ. Shape, four-sided prism or irregular, in most cases terminal walls at right angles to lateral walls. Walls thin, 1μ or less. All cells contain a dark granular protoplast with a thick ectoplasm. The nucleus is large, spherical, measuring 9 to 13μ in diameter. In some cases protoplasts contain starch. I found crescent-shaped grains which I took to be starch in the process of being broken down. Originally they were probably shaped like the starch-grains found in the medullary ray parenchyma cells. The resin cells can extrude their protoplasmic contents into the resin duct and bring about tylosis. (Plate 4.)

b. Parenchyma Cells (number measured, 20).

R, 13 to 28μ, average 18μ; *T*, 13 to 28μ, average 18μ; *L*, 60 to 225μ, average 120μ. Cell-wall thin, less than 1μ. Shape of cell similar to resin cells, but longer and smaller in radial and tangential width. Pits simple and as a rule oval or oblong. Cells contain living contents, that is, less granular than the protoplasts of the resin cells. Nuclei in most cases more or less elongated and smaller than the nuclei of the resin cells. (Plate 5.)

IV. Elements of the Protoxylem.

a. Spiral Ducts (number measured, 20).

R, 7 to 26μ, average 19μ; *T*, 8 to 28μ, average 19μ. Cylindrical, continuous ducts with spiral thickenings. The spirals are low, thin, and very close together. The middle lamella and secondary thickening layer both stain blue in anilin blue. The tertiary layer and the spirals are lignified and stain red in safranin. Pits are bordered, circular, and measure 9μ in diameter. They may be found on either the radial or tangential walls. The spiral ducts, in conjunction with parenchyma cells, form the leaf-traces. (Plate 5, 16.)

V. Elements of the Medulla, or Pith.

a. Pith Cells (number measured, 100).

R and *T*, 19 to 102μ; *L*, 65 to 276μ. Cells cylindrical, 4 to 8 sided prisms, or irregular. Walls thin and consisting of cellulose, or near the protoxylem they may be rather heavy and slightly lignified. The cells in center of pith slightly larger than cells near the protoxylem. Pits simple; their shape may be oblong, oval, or fusiform. The pits on lateral walls measure from 6 by 9μ to 12 by 16μ; pits on terminal walls 5 by 7μ. In the pith of very young stems the protoplasts contain starch. The starch is in the same form as that described in the medullary-ray parenchyma cells. Sometimes cells of older pith are plugged with resin. (Plate 5, 17.)

In some gymnosperms the very young pith contains heavy-walled lignified cells that are more or less star-shaped (idioblasts). I did not examine the pith just back of the growing tip, and, therefore, do not know whether they exist there or not.

ENTRANCE OF ELECTROLYTES INTO PLANTS.

The entrance of salts from the soil into the roots of plants is largely independent of the absorption of water by osmosis. The ions of electrolytes penetrate the colloidal walls of root-hairs and pass into the xylem or sap stream by diffusion at a rate mainly determined by their own ionic mobilities, by the action of other electrolytes present, and by the nature of the colloidal material in the walls and plasmatic material of the living cells traversed.[1]

The absorption of water can take place only by osmosis. If the living cells of the root, including the cortex and endodermis, be considered as the membrane in such an endosmotic machine, the nature and the rate of endosmose would be determined by the relative isotonic values of the liquid in the xylem or woody cells at the lower terminals of the system. To what extent the living cells exert an exudation pressure by which water is forced into the conducting cells of the root is not known. It is in any case an osmotic process in contrast to the action at the upper end of the system in the leaves where the initiation of movement is caused by transpiration from the outer surfaces of living cells in which the sap thus becoming more concentrated exerts an additional pull or osmotic attraction for water in the complex column in the wood cells. This may be taken to be the principal force operative in the ascent of sap, and in producing the reversible variations in thickness of the vessels and woody cells in trees.

The water and the electrolytes which enter the roots, passing the epidermis and entering the xylem will also receive material resulting from the breaking-down of the plasma, and carbohydrates which have come down from the leaves. Exchanges of many kinds will take place as the stream passes upward and comes into contact with the ray cells containing soluble carbohydrates. Electrolytes will pass into these and other cells at a rate determined by the state of the colloids in the walls, and additional carbohydrates may go into the upwardly moving stream, which ultimately reaches the transpiring cells in the leaf. The volume of water which thus passes upward in a stem during its development is relatively enormous, but the amount of solid material carried is small in comparison with the carbohydrates which are formed in the leaves and carried downward, being used in the construction of the trunk. The mechanism and path of this downward translocation has been the subject of much conjecture. The prevalent assumption that sugars diffuse downward through living cells or elements with gelatinous contents disregards some insurmountable

[1] MacDougal, D. T. The arrangement and action of material in the plasmatic layers and cell-walls of plants. Proc. Amer. Phil. Soc., 63, 76. 1924.

physical difficulties, as has been clearly shown by Professor Dixon. (Nature, 111, 236, 547. 1922.)

Some evidence as to the occurrence of sugars in the trunk of the Monterey pine is given in the present paper. Positive identification of the route of upwardly moving solutions is much more easily attained.

CONDUITS OF UPWARD-MOVING SOLUTIONS.

The path followed by a solution entering a root of a tree is very strict, so that a substance entering by one root may reach only a narrow sector of the shoot, as has been found by many investigators. Although the tracheids are connected laterally or tangentially, yet diffusion which would carry a solution around the stem is very slow, the system being set for upward conduction. It is of course in this direction that the tension set up by transpiration acts.

The layers which may serve as conduits would be determined by the condition of the contents of the woody cells and the membrane of the bordered pits. While some authors take the attitude that it is the wood of the previous year which may be followed by the ascending stream, several are seen to carry dye solutions entering the bases of cut stems, or by perforations in the trunk or into the cut ends of roots of the Monterey pine as described below.

The numerous tests with dye solutions to ascertain the layers through which solutions moved upwardly in stems were nearly all made about the close of the growing-season of the Monterey pine in 1923 and 1924. At this time of the year conduction is most rapid and the solution is apparently carried in the greatest volume in the wood of the previous year. Some movement also takes place in the third and fourth layers. The repetition of the tests in 1924, in July and August, the season for growth having ended by the first of May, was with material in which all of the wood layers had attained relatively greater seasonal "age."

The newly formed wood at this time was devoid of plasmatic material, except a few outer layers, and the liquid in the new wood cells was in effect a part of the continuous column which filled the older tracheids. The newly formed tracheids could not become the path of a direct upward current until the following year, when the new leaves will be joined directly to them. As will be seen by consulting the results of the experiments when cut stems were stepped into vessels containing dyes, the color was found to go into the outermost, most recently formed wood up for some distance. Diffusion or passage into this layer could take place through the wood cells of the rays, and the color never reached more than about half the height in the outer layer that it did in the wood of the previous year. The wood formed during two previous years is in direct connection with the leaves of this pine, while on terminals and in the shoots of young plants

leaves may be retained until the close of the third or even the fourth season.

As a new layer would come into conduction an older one would go out of commission. Any cause, however, which would operate to prevent the entrance of gas-bubbles into the older layers or which would cause their absorption, such as might occur in the basal part of the stem under pressure, would tend to continue the water columns in an older layer and such continued conduction has been observed in some stems absorbing fuchsin solutions.

The general program outlined has a variant in the terminal shoot and branches, especially marked in the Monterey pine, which may develop leaders 1 to 3 meters in length during a single season. A stem of this kind has a large pith and a hollow cylinder of wood of varying thickness. As it extends it forms the only path by which solutions from below may reach the tips. A terminal stem of this kind taken in July 1924 had finished growth in May. After being cut off it was stepped in a vessel of fuchsin for 24 hours. At the end of this time the pith was uncolored, but the entire shell of wood had taken the dye uniformly. The cambium and phloem remained uncolored.

Another experimental specimen included the internode next the terminal, which had the initial layer laid down during its development in 1923, and externally the layer formed in the growing season ending May 1924. The base of this cutting was stepped in dye and allowed to remain 24 hours. At the end of this time the dye had ascended in both layers of wood, but had not been carried in all parts of the layers. An uncolored strip lay between the two main shells, which probably represented the autumnal wood of 1923 which, with its small tracheids, would be less favorable to conduction than the larger cells previously formed and the tracheids of 1924.

Some distinctions as to the differential conduction capacity of the layers of various ages may be made with dyes which are true solutions and which will stain living material, passing cell-walls readily as well as plasmatic layers, and others with large colloidal particles which will not readily pass such membranes.

Orange G was used as having great penetrative capacity, also fuchsin S and acid stain, the colloidal particles of which are so large as to penetrate living cells very slowly while they may be carried along a sap stream in the tracheids very readily. These dyes were used in a solution of 1 to 1,000 in water.

A small tree about 2 cm. in diameter at the base was cut off, the top cut away, the branches taken off, and the remaining part, 1.8 meters length, set upright in a flask of orange G 1 to 1,000. Sixteen hours later the dye had gone up to a distance of about 50 cm., staining the three outer layers of wood, the third from the outside, presumably that of the early part of 1923, most strongly. The phloem was also

stained, a result to be expected from this dye which is one of the most rapidly penetrating tested by Ruhland and Kuster.[1]

A series of tests was arranged to unmask any other specializations in conduction which might play an important part in moving the sap stream. A small stem about 1.5 meters in length and about 2 cm. in diameter at the base was bent bow-shape, with the ends in a solution of orange G. All of the branches on being removed showed a stain about 35 cm. from the basal end and 20 cm. from the apical end in the same time.

While such tests would tend to show that the mechanism of the stem is adjusted for better conduction downward than upward, yet there are many conditions to be taken into account. Thus, in a freshly cut stem the tensions of the osmotic pull of the leaves would be present when freshly cut. The dye would be taken into the stem in accordance with the equalization movements. In fact, the introduction of dyes into the living stems may be used to show the direction in which solutions are moving in the complex column in the woody cells. In such an experiment with a tree on June 25, 1924, a hole 1 cm. in diameter was bored horizontally and tangentially in the trunk 2 meters from the base. A section of threaded brass tube was screwed into this hole and connected by rubber tubing with a filling bottle. Fuchsin S, which was acid, 1 to 1,000, was supplied. At the end of 120 hours the dye was traceable upward to a node bearing a half dozen vigorous branches 217 cm. from the point of application, the conduction being chiefly in the wood of the previous year.

Downward, the dye had diffused slowly through layers internal to that of the upwardly moving stream to a distance of 40 cm., at a rate which is seen to be more than one-sixth that of the upwardly moving stream.

The dyed portion of the stump was cut off and some cavities bored into the surface of the stump which were kept filled with fuchsin for a day. At the end of this time the dye, which had gone down for a few centimeters in all of the layers, had stained the fourth layer from the outside downward to a length of 40 cm.

It is to be seen that the dye had moved upward at what may be taken as an average rate for the end of the growing period and that the downward movement was greater in inner layers in which the movement was at a minimum. When the tree was cut off and the basal section freed from the osmotic pull of the leaves, the dye had diffused downward as far in one day as in five days when the osmotic pull of the leaves was carrying solutions upward.

Another test giving illustrative results was carried out as follows:

On May 27, 1923, a young pine tree 2.5 meters high, 2 cm. in diameter at the base, was cut off at the base and set in fuchsin S at 5 p. m.;

[1] Ruhland, W. Studien ueber die Aufnahme von Kolloiden durch die Pflanzliche Plasmahaut. Jahrb f. Wiss. Bot., 51, 376–431. 1912.

16 hours later (the next morning) the color had risen to a height of 60 cm., or at the rate of about 4 cm. per hour. The development of the stem had been excentric, due to the fact that it was leaning uphill away from an overshadowing tree, the layers being less than 1 mm. in thickness on this side and 1 to 2 mm. on the other side, from which most of the branches arose. The dye had risen farthest in the thin layers, not showing higher than 25 cm. on the side composed of the heavier layers.

A smaller tree with thin layers, attached to the barometer, was set in a fuchsin solution, with the result that the dye showed to a height of 30 cm. The mercury was pulled up a few centimeters, then fell back, but no air-cushion had been formed at the base of the stem.

A stem 15 cm. at the base, cut at $9^h 30^m$ a. m., warm and sunny, at $3^h 30^m$ p. m. had conducted the dye 60 cm. in 6 hours. The color had also diffused laterally on one side of the stem, staining the cambium at this extreme length. Another stem put in the dye at 4 p. m. had conducted the color 100 cm. by 8 a. m., which was at the rate of 6 cm. per hour.

Ordinarily solutions of electrolytes enter the plant through the endodermis of the root, the water being pulled by osmotic action across this continuous plasmatic layer, while the electrolytes enter in accordance with a rate determined by their own ionic mobilities and influenced by various absorptive processes. This action is such that the larger roots, in which the streams from the rootlets gather, show their greatest enlargement during the daytime or warmer period of the day. Once the electrolytes are inside the non-living cells in addition to their own diffusive action, they are carried along with the ascending sap stream at rates comparable to those found by the use of dyes in experimental tests.

The behavior of dyes entering through the roots may be illustrated by the following observations:

A pine tree, 4 cm. in diameter at the base and 2.5 meters in height, was selected and the soil removed to uncover the largest lateral root, which was 2 cm. in diameter where it joined the stem. This was cut off 14 cm. from the base of the stem and a section of rubber tubing coupled on. Early in July 1924, a cell and pressure column of mercury were provided, the system being filled with acid fuchsin, so that the surfaces were not allowed to get dry; 120 hours later the tree was taken up, bisected, and the course of the dye followed. The color had been forced into the entire cross-section of the root and, as the center of this root was made of wood which had originated when the stem was young, the color was conducted to the center of the base of the stem. A pressure of 1 to 1.5 atmospheres was maintained on the cell containing the dye solution and the stain was found to have gone upward through the greater part of the length of the stem in the radii above the

root junction. The path of the solution was distorted by the complex of wood cells at the bases of the stumps of two large branches cut off earlier in the season. Furthermore, it was distributed more than three-fourths of the way round the stem in the nodes formed during the preceding two years. In any part of the stem more than 3 years old the dye was heaviest in the second layer, though found in the third, while the wood of the present year was stained, the dye passing into the cambium in the upper part of the shoot.

The experiment was repeated with a larger tree on July 23. This tree stood near the laboratory, was 6 meters in height, 6.5 cm. in diameter at the base, and had numerous branches. A small lateral root immediately below the surface which had a diameter of about 1 cm. at its juncture with the stem was cut off a few centimeters from the stem and fitted with a tightly clamped rubber hose and a cell containing fuchsin S under pressure, which was adjusted between 1 and 2.5 atmospheres.

A day later, about 200 c. c. of the dye solution had been forced into the stem. The system was refilled and the pressure adjusted to about 1.5 atmospheres. The amount of liquid entering the stem on the second day was less than half that on the first day.

The tree was cut at the end of 120 hours, when about 300 ml. of the solution had gone into the stem through the root. The dye had passed into the center of the stem and then started upward in 7 layers of the wood, not including the outermost and innermost layers. The tree was thus made to be 9 years old. Within a meter from the base the dye had diffused into the outer layer. Within 2 meters it had stopped in all but the first, second, and third layers. Finally, in the last half meter, the dye reached its farthest point from the place of entrance, 2.6 meters in the outer layer only. In this and other cases in these experiments the wood of shoots which bear leaves on the surfaces apparently becomes capable of conduction shortly after the end of the growing-season. The terminal parts of the shoots of this pine showed conduction in both layers of wood when only two were formed in nearly all instances.

The node above this was taken for an estimation of the reducing sugars in the first and second layers. It was seen that the darker layers of brownish wood were well defined and broad. The strict ascent of the dye to tracts of wood directly connected with the conduits below was seen here about as described in another section of this paper.

The terminal part of the tree, about 2.5 meters in length and including three long internodes and the short leader developed in 1924, was now cut and stepped in a solution of fuchsin at 11 a. m. The preparation weighed 4,800 kg. At 8 o'clock the next morning it was found that the shoot had taken up 350 ml. of the solution and that the

shoot had not changed in weight. The color had just passed the bases of a heavy whorl of six branches 80 cm. from the base, which probably carried more than half the transpiratory capacity of the preparation. Half this length the dye had gone up in all of the layers. Above this, the outer and innermost layers were uncolored, the second, third, and fourth being stained. Above the node which carried the heavy whorl of branches the dye was seen only in the second layer, or in the wood formed in 1923. This was also the innermost layer.

It would seem, therefore, that the outer layer in this part of the stem had not yet assumed the condition in which colloidal material might pass through the wood cells readily. Lower down in the stem, about midway of its entire length, the dye was carried most readily in this outer layer.

The fuchsin solutions used in these experiments are acid, but as used the acidity does not give the most color. The possibility therefore existed and must be taken into account in all of these tests that a layer might appear more deeply stained by reason of its resident acidity, which would intensify the color taken in and produce an effect which would not show the relative conductive action of the different layers. Surfaces of stems in which comparisons of color in the layers were being made were therefore lightly moistened with a weak acid solution. The results were not changed in any instance in tests made during the summer months, but it is possible that the relative depth of staining of different layers might be altered during the growing-season by bringing them up to the maximum acidity of the dye.

The matter of amount of transpiration loss and intake of water by the cut end of a shoot is one which has received much attention. Another shoot of about the same size as the above and weighing 4,700 kg. was set in a dish of water and placed on a balance. This was arranged late in the afternoon. At 8 a. m., 16 hours later, the shoot had taken up 135 ml. of the solution but showed a net loss of 70 grams, indicating a total transpiration of 205 ml.

A smaller tree taken at the same time as the last named, late in July, showing 7 layers of wood in the basal portion, had a slender root coming out near the surface of the soil. This was immersed to a length of 20 cm. in a cylinder of fuchsin for 120 hours. No pressure was applied. The dye was traceable for a distance of approximately 1 meter. The character of the stained portion of the stem was unusual. The dye started upward in a thin, strictly defined layer, the third from the outside. The first node, 40 cm. up the stem, was reached in the second and third layers. Above this node the color disappeared from the third layer and was found in the outer one and in the second layer for a distance. Its furthest extension was traceable in the outer layer, so that the dye could be seen when the bark was stripped from the stem above the node. The conditions suggest that the outer layer

had assumed the mature condition in which such colloidal solutions could pass readily.

The greater number of tests of the capacity of different layers of the trunk of the Monterey pine for conducting dyes in 1923 and 1924 were made near the close of the growing-season or later, when the tree was in the autumnal condition, in which the transpiration stream was at a minimum. A review of all notes made on this subject discloses the fact that in parts of the stem more than 2 years old, the greatest upward movement takes place at this stage in the second, third, and fourth layers from the outside. Occasional tracts are found toward the center in which conduction is also marked. Upward conduction in terminals, or in parts of the stem 2 years old, may take place in the outermost or most recently formed wood.

RESISTANCE ENCOUNTERED BY TRANSPIRATION STREAM.

According to Dixon's estimate, the force necessary to raise water to the top of a tree would be equivalent to the pressure which would be exerted by a column of water about twice the height of the tree. The filtration resistance encountered by water passing upward through the two or three layers in which the sap chiefly ascends in the Monterey pine would obviously depend upon the size of the tracheids, especially their diameter, the area and number of the perforations in the pit-membranes, and the speed of the current, which, of course, would be determined by the rate and amount of transpiration.

The tracheids are found to reflect in some measure the transpiratory conditions of the individual. Thus, a plant with a high rate of loss of water would be found to be furnished with conduits showing a minimum resistance, and plants with a low transpiration-rate generally have smaller conduits. Hüber found that the average flow through branches 1 to 4 years old of deciduous trees encountered a resistance of 0.2 to 0.4 atmosphere per meter length. A tree 10 meters in height would require a force of 2 to 4 atmospheres to raise water to the top. Hüber estimated that the speed of the transpiration stream in the larch was such that a tension of about 7 atmospheres would be required to deliver the necessary amount of water to the evaporating surfaces; and tensions of this magnitude were demonstrated in this tree.[1] These calculations were based on the action of branches, in which the flow went through the entire cross-section of the stem. In older trunks of the Monterey pine the conduits would be in the form of shells 1 to 4 layers in thickness. The cross-section of these conduits would increase with size of the trunk and would be found

[1] B. Hüber. Beiträge zur Kenntniss der Wasserbewegung in der Pflanze. II. Die Strömungsgeschwindigkeit und die Grösse der Wiederstände in den Leitbahnen. Ber. d. Deut. Bot. Gesell., 42, 27. 1924.

by subtracting the area of the cross-section of the woody cylinder internal to the conducting tract from the total area of the cross-section of the trunk. The differential rates in the various layers are illustrated by the results described in the preceding section. When a stem or trunk is cut off and stepped into a dye solution, the color is found in all of the woody layers after a few hours. After the accumulated tensions have thus been equalized, the movement of the colored liquid will indicate directly the rate of flow resulting from osmotic pull of the transpiring leaves against resistance of the conduits.

That the tensions necessary to induce the flow are comparatively small is indicated by the fact that when a pump is attached to a branch replacing some of the leaves, the fraction of an atmosphere additional tension set up causes a marked increase in the rate of flow of material through a stem. The terminal of a pine tree 6.5 meters high with many branches was cut away and the suction-hose of a Nelson air-pump attached in its place. An acceleration of the ascent of the sap as denoted by the movement of dye solutions resulted. Such an arrangement entailed the lessening of the transpiration total by the amount which would have been thrown off by the renewed shoot and the substitution of the action of the pump, which kept a suction of 0.6 to 0.7 atmosphere on the stump. This was sufficient to replace the action of the shoot and to give an increased action. The actual net increase of the pulling power of the system would be comparatively small, perhaps not more than half an atmosphere more than that of the normal shoot, yet the effect of the upward movement was very marked.

The results of a number of experiments in the use of the pump were in general agreement with the above. Obviously, the effect of the pump would be relatively greater with decreasing length of stem. Dye "in quantity" was pulled through a pine stem over a meter in length in 5 hours, while a length of only 10 cm. had been traversed in a leafy stem in the same time. Both stems were more than a meter in length and may be estimated to have offered a filtration resistance of less than half an atmosphere to the normal rate of flow. Under such conditions the use of suction amounting to nearly an atmosphere resulted in a greatly accelerated flow.[1]

Bode was not able to detect any changes in the dimensions of stems as the result of suction by a pump, or a pull of one atmosphere; but the method given above would be a much more delicate test of the effect of suction on movement of liquids in stems.

It is not clear whether the increased resistance of the tracheids of a dead trunk is due to the presence of air-bubbles or to the appression of the tori against the opening in the bordered pit. Perhaps both of

[1] MacDougal, D. T. and F. Shreve. Growth in trees and massive organs of plants. Carnegie Inst. Wash. Pub. No. 350, 1924, pp. 29–34.

these conditions are operative. Among other experiments on the effects of defoliation it was found that the removal of the leaves from a tree in the autumnal resting condition resulted in the death of the tree and that some fluctuating or reversible variations in the diameter of the stem ensued after death, which would be indicative of the persistence of a continuous water column under tension, for many days after death.

Another tree defoliated in the autumnal condition, when stepped in dye in the following June, showed but little conduction of fuchsin, which ascended less than 5 cm. in two days. When the upper end of the trunk was connected with the air-pump, no appreciable effect was seen, even when sections less than a meter in length were tested. Some dye was pulled up into the large cavity left by the disintegration of the pith in short sections, however.

A tree about 2.5 meters in height (27*b*), which was defoliated in January, at the beginning of the growing season, had a few small tufts of undeveloped leaves on the ends of the uppermost branches in July. The sugar-content of the stem was 25 per cent below normal (see p. 77), and the moisture content of the stem was less than 36 per cent as compared with that of a normal stem at 60 per cent. Dye ascended only a few centimeters when the base of the stem was stepped in a vessel containing fuchsin. The use of the pump was followed by results as described above. The water column was probably broken, and it is also probable that the bordered pits were in a condition in which they offered increased resistance to filtration. Solutions would have been pulled through living stems of similar size at a very rapid rate.

COMPARATIVE CONDUCTION UNDER SUCTION OR TRANSPIRATIONAL PULL AND UNDER PRESSURE BASALLY APPLIED.

The ascent of solutions may be assumed to take place most readily in tracts in which the cohesive column under tension is subject to the least resistance from membranes and from air-bubbles. The conduction of dyes at lesser rates in various layers of wood may be ascribed to the presence of air-bubbles or to the disposition of the tori in the pits which would partially close them. It must be noted, however, that when a cut stem is stepped in dye and the color appears in various layers for short lengths, that the color may pass from one layer to another by way of the ray tracheids in the case of colloidal liquids, while true solutions might diffuse through thin membranes.

The cohesion tensions set up in small trees of the Monterey pine may not be very great, since the movements of solutions in such stems may be materially and strikingly modified by the use of an air-

pump, which, replacing the leafy terminal, gives a net increase not more than 0.5 atmosphere. The use of such a pump would not pull gas-bubbles through perforations in pit membranes. The reduced pressure on these air-bubbles would, however, lessen their viscosity.

The application of dyes under pressure to the cut ends of stems would tend to increase the flow in tracts with the least filtration resistance. Such pressure would tend to compress and increase the viscosity of air-bubbles if present. The comparative action of the tori would be problematical. That some differential effects may be produced by pressure basally applied to the stem, as might come from root-pressures, was suggested by results of the first two tests dealing with the matter. A young pine tree 22 to 25 mm. in diameter at the base was cut and quickly fitted with a cell containing fuchsin and attached to a pressure system including a vertical tube with mercury. The pressure was maintained at 0.7 to 1.4 atmospheres for 2 hours. The dye was forced upward a short distance in all of the wood, but much farther in the fourth layer from the outside, next farthest in the second layer from the outside or wood of 1923. The terminal and all of the branches, except two small ones with a few dozen leaves, were removed in order to eliminate transpiratory pull at the beginning of the experiment. After the above results were noted, the stained part of the stem was cut away and the base of the remaining part was stepped in an open vessel of dye. The color now ascended in the second layer from the outside twice as far as in any other, even with the reduced transpirational pull.

On August 21, 1924, another set of tests was applied. A stem 12 mm. in diameter at the base, 2 meters high, took the color up in all layers, including the outermost one, most recently laid down, to a distance of 15 cm. Above this the color was not carried by the two outer layers, but had gone up the stem in the third and fourth layers to a height of about 40 cm. in 5 hours. A similar shoot of another tree was fastened to a cell containing fuchsin on which a pressure of 1.6 atmospheres was applied by a mercury column. The color ascended 110 cm., or nearly three times as far in the same time as in the stem in which the transpirational tension only was present. The dye had ascended in all of the woody layers except the innermost to about 50 cm. Above this it became restricted to the two outer layers. The dye had gone up in a narrow sector only of the outermost layer in this upper half of the part penetrated, while the second layer, or the wood of 1923, was stained as a fairly complete shell. So much of this stem was colored that it could not be used for a test without pressure. The first stem was now fitted to the pressure system. Two hours later the dye had ascended to a height of about 45 cm. in the second layer formed in 1923, in which conduction was slowest without pressure, while all the wood was colored for about one-third

this distance. It is to be noted that the addition of this basal pressure to the transpiratory tension had resulted in an acceleration of the sap from a conduction rate of about 8 cm. per hour to 22 cm., which high rate was identical with that shown by the other stem when under pressure.

A larger stem, 3 cm. in diameter at the base, was cut to a length of a meter, the basal end stepped into an open vessel of dye, and the upper end inserted into a connection with a rotary pump, which soon set up and maintained a column of mercury 750 mm. in height. This pressure was maintained for an hour and a half, when the pump was stopped. Two hours later about 100 c. c. of sap had collected in a flask in the exhaust line, which was found to be slightly acid (pH 6.8). This sap had been displaced by the dye in all of the wood of the lower part of the stem except the fifth layer. The sixth layer carried the color half the entire length of the colored part. The outermost layer was stained, except two sectors, to half the length of the stem, when it diminished. The upper half of the meter length of stem was stained only in the wood of 1923, which was set off from the other layers very sharply by the color.

The diversity of pattern made by the dye in the ascent of these stems is one which may be explainable on the basis of the differential stages in which the wood was found. The wood of 1923 was, on the whole, the readiest conducting layer under both suction and pressure basally applied.

Acceleration under basally applied pressure was about the same in the two stems tested. The dye was carried to a distance of 90 cm. in 4 hours in the stem connected with the pump, during 2 hours of which time the pump was not operated. This action is in accordance with observations in which stems attached to a potometer are seen to continue the pull on the water column for some time after defoliation. It seems highly probable that such action is dependent upon the expansion of the wood cells and also of living cells when freed from the pull on the upper end of the system. Such expansions are invariably noted in trees of Monterey pine during the first day or two after defoliation.

An extension of these experiments at this time seemed to promise results of value, and on August 23, 1924, another small pine tree 2 meters in height was cut off near the base and the lower end set in a solution of erythrosin, 1 part to 1,000 of water, while the terminal was cut away and the stump connected with a Nelson pump by a section of rubber hose. The excised terminal was placed with its base in another dish of the dye. During the next 24 hours the pump was operated 1 hour, maintaining a pressure or suction of about 740 mm. Hg. The color had been drawn up to a distance of 30 cm. in the fourth layer and was found half that distance in all of the layers

in the section attached to the pump. The terminal showed all of the layers colored for some distance, but conduction has been greatest in the second layer, the wood formed in 1923.

A test was now made using a solution of methyl blue, which does not penetrate living cells readily, and in which the particles of dye are of a size in which they supposedly pass through openings in membranes only. A tree similar to the above, 3 cm. in diameter at the base, was cut off and stepped in a vessel containing methyl-blue solution. The terminal was cut at a point where its diameter was 2.5 cm. and connection was made with a Nelson pump. The terminal was also stepped in the vessel of dye. These preparations were made at 4 p. m. on August 24, but the pump was not started until 8 a. m. the next day, when it was operated to maintain a pressure of 740 mm. Hg for 3 hours. At the end of this time both parts of the shoots were dissected. The blue had ascended the basal part of the tree under the action of the pump for a distance of only 20 cm. in the external layers, including the outermost or newest wood. Color was found in all of the wood for half the length noted. The dye was seen to a length of 15 cm. in all of the wood of the basal part of the terminal and above this it had gone as much farther in the two outer layers of wood.

The test was repeated with a similar tree. The lower part of the stem under the action of the pump showed the dye in all layers to a height of 35 cm., where it disappeared from the outermost layer, or newest wood, and then reached a distance of 48 cm. from the base in the fourth layer. The upper part of the shoot with its heavy leafage standing in a dish of the dye showed the dye in all of the layers to a height of only 15 cm. Above this the dye had ascended 15 cm. farther in a narrow sector of the second layer.

The colored parts of both stems were cut away, the one attached to the pump was freed, and both were set in a vessel of the dye for 48 hours. The one to which the pump had been attached in the first part of the experiment had carried the dye to a height of 40 cm. in all of the wood and 20 cm. farther in the fourth layer in a narrow sector. The terminal included but three layers, and the dye had gone up 30 cm. in all, and to a distance of 15 cm. farther in the second layer, or wood of 1923.

Another pine, about 3.5 meters in height and 3.5 cm. at the base, was cut in two, the basal section being connected at the upper end with the pump and the lower end set in methyl blue. The base of the upper part of the shoot with many leaves was set in an open vessel of dye on the eve of the 25th. The pump was operated for 3 hours on the morning of the 26th.

Dissections were made of the lower ends of both preparations at 2 p. m. The dye had gone up about 30 cm. in the basal part of the main stem in the outermost layer and in the third layer in a narrow

sector. The color was seen in all of the wood for about half this distance, in which the wood was deply stained. The blue had gone up sparingly in the upper part of the shoot to about the same distance as in the basal part, which was attached to the pump. This deeper staining may be definitely connected with the effects of the pump. The greatest distance had been reached by the dye in the second layer. All of the layers were stained in the first 10 cm. above the base. The stems were again set in the solutions and the pump operated for nearly 4 hours at intervals. At 8 a. m. on the 27th about 40 c. c. of liquid had been collected in the first flask nearest the top of the shoot in the system leading to the pump. About 150 c. c. of liquid had been taken up from the dye solution. Some evaporation had taken place from the half dozen leafy branches on the stem and doubtless some water had been pulled through the pump. The basal part of the stem was stained in all layers, the autumnal wood remaining as whitish stripes separating the layers.

The dye had been carried nearly to the upper end of the stem as described above through a length of about 1.8 meters. It was observed that the extraction of sap and the upward movement of the dye did not begin at once with the action of the pump, but only after about 3 hours of operation, when the sap came away in quantities and the dye was seen to pass rapidly upward near the surface of the stem.

DOWNWARD MOVEMENT OF SOLUTIONS IN WOODY STEMS.

Nothing in the foregoing experiments might be taken as indicating the path followed or the rate at which the complex organic material originating in the leaves finds its way to all external parts of the stem and to the extremest root-tips. When it is considered that the distance to be traversed from the leaves of a Monterey pine to the extremities of the roots may be 20 to 40 meters, and that organic material in the redwood mentioned in a previous paragraph must traverse a distance which can be no less than 35 to 40 meters from any green cell to the base of the trunk, and that some of the rootlets are 10 to 15 meters from this region, it is evident that any scheme which calls for the diffusion of material rather than by a current presents a problem of transportation in which it would seem almost impossible to get the material actually used through the cells and down the stem at a rate consonant with the facts, as has been pointed out by Professor Dixon.[1] Dixon and Atkins have also set out clearly the manner in which sugars may pass into the tracheary elements and be carried upward.[2]

[1] Dixon, H. H. The rôle of transpiration current in the transport of substances throughout the plant. P. 51 of "The transpiration stream," The University of London Press. 1924.

[2] Dixon, H. H., and W. R. G. Atkins. Osmotic pressures in plants. Notes from the Botanical School, Trinity Coll., Dublin, 2, 275, 294, and 335. 1916.

The studies of Dixon and Atkins were made on dicotyledonous stems in which elongated tracheary elements were present and the conducting systems were complicated.

The present studies were made chiefly with *Pinus radiata*, a tree in which the conducting system is made up of tracheids of well-known structure, and the behavior of this tree in the matter of growth and in changes of volume inevitably closely connected with the movements of solutions have been the subject of extended and accurate measurements. The problem, therefore, has not been dealt with generally. Attention has been directed to the determination of the manner in which sap ascends and organic material moves in such a coniferous stem and the chief comparisons have been made by observations upon *Sequoia* upon which similar measurements are also being made. Whatever scheme of operation may be found to prevail in these stems, it may be taken for granted that modifications would be seen in the ascent of sap in trees in which the conducting tracts include other and more complicated structures.

The path of the upwardly moving material has approximately been defined and located in the Monterey pine by the use of fuchsin solutions. Not only is the upward movement known to be chiefly in layers of wood 1 and 2 years, or 3 years, old, but solutions are confined to the sector of wood into which they are first directed from a root or by artificial injection. The rate of lateral diffusion is very low. Material with particles as large as those of fuchsin passes upward in tracheids at a rate from 100 to 1,000 times as great as that by which they may pass radially toward the center or the periphery of the stem.

The upwardly moving current contains not only the electrolytes which have entered the plant through the root surfaces, but also carbohydrates, seasonal occurrence and distribution of which have been defined by Dixon and Atkins. These carbohydrates originate in the green organs of the plants and must find their way downwards to all parts of the plant, some being used in construction and respiration. A part of this material diffusing through the thin walls of the medullary cells or passing through the ray tracheids finds its way into the conduits and is carried upward, and the rate of the movement may be indicated by that of the dye solutions as described.

The determination of the path by which the sugars first descend the stem has long been a most perplexing problem. After repeated demonstration of the fact that upwardly moving streams do not usually follow the recently formed wood, but are strongest in the wood cells of the previous year, it seemed obvious that in this new wood was a possible conductor of carbohydrates and that the matter had not been fully tested.

A test of the sugars in the first and second layers of the small tree which had been injected with dye through a root as described on

page 18 was made in mid-July. Material was taken from a node above that in which the color had reached its farthest extension in the outer layer. This was done within a few minutes after the tree was cut to avoid error which might arise from diffusions following the disturbance of the transpiration stream. The main part of the outermost layer, which was formed earlier in this year, was cut into thin shavings and extracted for the picric-acid reduction method of Thomas and Dutcher, which gives the amounts of reducing sugars present only. The brown outermost part of the second layer was removed and the remainder of this was taken for a similar estimation. These layers were 4 to 5 mm. in thickness and were easily separable.

It was found that the proportion of the reducing sugars to the dry weight of the second layer was 0.007 per cent and in the outermost layer 0.204 per cent. Glucose and maltose probably formed the greater part of these carbohydrates, although no attempts were made to determine the constitution of the sugar content more exactly.

Dixon and Atkins note that sucrose generally preponderates in the carbohydrates of transport, although sucrose and the reducing sugars were more evenly balanced in *Ilex* and *Fagus*, and it was noted that sucrose was entirely absent in two specimens of the last-named tree.[1]

Further discussion of the factors to be taken into account in the determination of the mechanism of the movement of organic material from the leaves toward the root is to be found in the section following.

TRANSPORTATION OF MATERIAL.

The facts known concerning the actual movement of water from the root-system to the leaves of the Monterey pine afford a basis for an adequate explanation of the upward movement of material in the xylem, although this conclusion has recently been contested by Professor O. F. Curtis. As will be shown, Curtis has failed to take into account all of the facts concerning the part and conduction of solutions from the roots to the leaves. Citing the results of Muenschner to the effect that there is no direct relation between water absorption and salt absorption and assuming that the movement of water upward through the xylem would be through a series of membranes, and also taking into account the results of a number of very ingenious experiments in girdling, Professor Curtis favors the suggestion that the mineral substances derived from the soil are carried chiefly in the phloem.[2]

[1] Dixon, H. H., and W. R. G. Atkins. Osmotic pressures in plants. IV: On the constituents and concentration of the sap in the conducting tracts and on the circulation of carbohydrates in plants. Notes from Bot. School of Trinity College, Dublin. 2, No. 6. April 1916. See also Atkins, W. R. G. Researches in plant physiology, p. 206. 1916.

[2] Muenschner, W. C. The effect of transpiration on the absorption of salts by plants. Amer. Jour. Bot., 9, 311. 1922.—Curtis, O. F. The effect of ringing a stem on the upward transfer of nitrogen and ash constituents. Amer. Jour. Bot., 10, 361. 1923.

The lack of direct connection between transpiration and the taking up of electrolytes from the soil would be in accordance with well-established facts, although some indirect connections between absorption of such material and of the amount of water-loss may prevail. The rate at which ions enter the root and cross the endodermal membrane is determined by their own ionic mobility and by the colloidal condition of the membrane as altered by the action of other ions present in interferences or antagonisms. The amount of water which would enter the root would be determined to some extent by the condition of the endodermal membrane resulting from the complex action of the entering ions.[1] It is also to be said that with the electrolytes entering the roots at rates largely independent of what happens osmotically at the same place, the amount of water which might be taken and carried up to the leaves by the transpirational pull might vary widely without affecting the so-called absorption of mineral nutrients.

It is extremely hazardous to apply conclusions derived from a study of the mechanical arrangements for the conduction of liquids in one plant to explain the movement in another. Professor Curtis says:

> "If there is an actual mass flow of water in the transpiration stream without passing filtering membranes, a flow as through a pipe in an ordinary water system, if nutrients are carried in this stream and the associated living cells do not remove them, then the rate of transpiration would, of course, influence the amount of nutrients reaching the transpiring tissues."

The perforations in the pit membranes of *Sequoia*, the Monterey pine, and other conifers furnish the conditions for an actual movement of water up in a complex column to meet the first condition mentioned, but the supply of electrolytes in this flow will be determined independently, as described above. Likewise, as the electrolytes come into the transpiration stream by their own action, they pass out of it into living cells of the xylem and also into the growing elements by their own translational velocities modified by the character of the colloids through which they pass and which they also modify. Combes found that the ash of leaves of ringed trees of *Pinus excelsa* had a higher ash content than those of normal stems according to Curtis, which could only denote that for some reason the electrolytes had not diffused out of the stream into the living cells contiguous to the conduits up the stem. This result was directly opposite to the effects found in the privet, peach, and lilac. These are types with short stems and with long open vessels as contrasted with the pine, in which the conduits are wood-cells of an average length of a few millimeters (4 mm. in Monterey pine).

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[1] MacDougal, D. T. The arrangement and action of material in the plasmatic layers and cell-walls of plants. Proc. Amer. Phil. Soc., 63, 76. 1924.

The conduits of the Monterey pine which carry the transpirational stream upward include the wood of the previous two years, which is ordinarily in direct connection with the leaves of the current year and of the year preceding. In some parts of the shoot the leaves are held for a longer period and good conduction may take place even in the fourth layer. Stems stepped in vessels containing dye carry it upward at a slow rate in almost all of the wood in this tree.

The outermost layer of wood or that formed during the present year is not connected directly with the leaves which developed early in the year and which are connected directly with the wood of the previous year. Hence the transpirational pull does not act directly on this layer, except perhaps in shoots of young trees. Some pits offer openings for the passage of liquids from this layer into the wood of the preceding year, however, so that the cohesion tension in the older wood would be communicated to this wood. The contents of the young wood might in this way be in a state of tension by which it would be drawn slowly out of the older wood into the transpiration stream. In the Monterey pine the new wood is heavily charged with sugar at certain times. The amount present in the new wood was found to be 30 times as great as the amount in the wood of the previous year in samples taken in July, when calculated against the dry weight of the material of the two layers. The presence of the sugar in this proportion is not conclusive proof that the outermost layer, which has not yet become directly connected with the leaves, is the conduit by which these substances move downward in the stem. The outermost wood is, however, not the path of an upwardly moving current in the normal tree. All of the wood cells of the outer layer except those most recently formed are clear of plasmatic contents and contain water with substances in solution and with particles of resinous material floating in the liquid or attached to the walls or pits. Diffusion might take place between these cells and the cambium or living cells external to them or the living ray cells. It is also to be noted that while all wood cells of this species are characteristically devoid of pits in the tangential walls by which movement of liquid might take place radially in either direction, yet the longitudinally arranged wood cells are in direct connection with the wood cells of the rays which do run radially. The new wood being always filled with liquid, this solution forms a part of the continuous water column which is connected with the leaves. The tension exerted on the water in the wood of the previous two years would also extend to these cells. Consequently the sugar in the most recent wood may move toward the older wood by the tension in the older wood as well as by osmotic action in the parenchyma cells of the rays from which this material might pass into the older wood. The character of the daily reversible variations in diameter would suggest that this external layer participates in the

contraction due to increased tension during the day and expansion following lessened tension in periods of lessened transpiration.

The connection of the outermost wood with the layer of the previous year by the ray tracheids would also account for the radial conduction of dyes into the outer layer in the experiments made for the purpose of tracing the conducting tracts. Dyes are carried much further from the base in the second and third layers, but they may also appear in other layers, both external and internal, being carried radially through the ray tracheids. It is to be noted, however, that the height to which the dye appears in the outermost layer is generally not more than half that in which it shows in the main conducting layers. The rate at which the color passes out through the rays is therefore much slower than that at which it goes upward in the stem. The upward movement is one which is due to the direct pull from the transpiring surfaces of cells in the leaves. The passage of the dye into the outer layer would be mainly by diffusion, although the liquid in the wood-cells of the outer layer is in connection with that of the main column and must to some extent be under some degree of tension communicated to it from the main column.

When a solution is sought for the problem as to the manner in which carbohydrates move down the stem of the Monterey pine, certain facts stand out prominently. First, the maximum concentration of reducing sugars is in the outermost layer; secondly, the region immediately above a girdled zone is one in which carbohydrate accumulates in the outermost layer of wood, as if its downward diffusion was stopped; thirdly, the amount of growth in this region is greatest and continues longest in a girdled tree.

No accurate information is available as to the condition of the perforations in the membranes of the bordered pits in the new ray cells and most recently formed wood. If these openings are cleared the continuous column of water in the vertically placed tracheids would connect directly or completely. If, on the other hand, these openings have not yet been cleared of mucilages, the passage of the dye into the outermost layer through such membranes would be simply by diffusion.

An attempt was made to ascertain some of the conditions of the diffusion of sugars from the outer layer of wood. An estimation of the proportion of sugar in the two outer layers of a branch of a normal young tree in September showed that the sugar present amounts to 0.05 per cent of the dry weight. When the two layers were separated, the outer layer was found to contain an amount of sugar equivalent to 0.146 per cent of its dry weight and the second layer 0.128 per cent of its dry weight in sugar. Early in the summer the disproportion was very much greater. This disparity, which was as 1 to 30, may be ascribed to the fact that sugar diffusing into the second layer traversed

by the main transpiration current was carried away quickly. In September the lessened transpiration stream did not carry away the sugar so rapidly, with the result that the second layer had nearly as much sugar as the outer layer.

Methods for tracing the movement in the outermost or most recently formed wood were not developed until late in the season of 1924, at which time marked retardations of movement might be expected. The only results which may be communicated at this time are those secured by cutting into the outer layer and placing a few drops of fuchsin solution in the slit, which did not extend so deep as the second layer. In some cases the dye moved downward 6 cm. in 24 hours, staining the entire layer, and upwards an equal distance. In the last case tested before the completion of this paper the dye in such an experiment moved downward a distance of 4 cm. in the outer layer and upward 10 cm. The use of this dye or of some readily recoverable reagent in a tree like the Monterey pine, in which the layers are thick and set off sharply, promises much in the way of evidence upon the capacity of the outermost layer, which has not been brought into the system in which sap moves upward to carry organic material in any direction. Furthermore, it is highly probable that whatever movement does take place in this layer, it will be found to be affected by or determined largely by the relation of the liquid in the layer to the cohesive column of water in the second, third, and fourth layers, which is in direct connection with the menisci in the outer parts of the walls of the transpiring leaves.

CORRELATION OF REVERSIBLE VARIATIONS IN STEMS WITH TRANSPIRATION AND UPWARD FLOW OF SOLUTIONS.

The extensive dendrographic records of the Monterey pine with the attached notes furnish material upon which the relations between the volume of trunks and stems and the balance between transpiration and absorption of water may be estimated at any time and also followed throughout the seasons. It is possible also to estimate the specific action of some external agencies that affect the water relations of plants upon the basis of the information included in the records.

A number of experimental arrangements of material have been made in which variations in the action of the shoot have been induced, yielding evidence of value in the determination of the system of translocation of material which exists in the plant. Some of these operations have been described in the preceding section, but may be cited in briefer form in the following pages.

The Monterey pine grows in a climate which includes a winter rainy season, with growth usually beginning in January, February, or March. Enlargement of the trunk and elongation of the shoots continues until

the depletion of the soil moisture reaches a point something above 6 per cent, when it ceases. Young trees growing in moist locations may continue activity through the entire summer. It is fairly certain, therefore, that the growing-season of the mass of the species is limited by the soil-moisture supply. Irrigation during the dry summer season will cause renewed activity in trees which have come to rest in dry locations.

Precipitation during the summer months of June, July, and August is negligible. Occasionally heavy rains early in September result in an increase in soil-moisture which, with the high temperatures of that and the next month, may cause an amount of growth or wood-formation greater than that which may have taken place in the usual January to June growing-season.

The daily reversible variations of the trunks of trees have an amplitude near the minimum in the autumnal or resting-period of the tree immediately preceding the beginning of seasonal activity in growth.

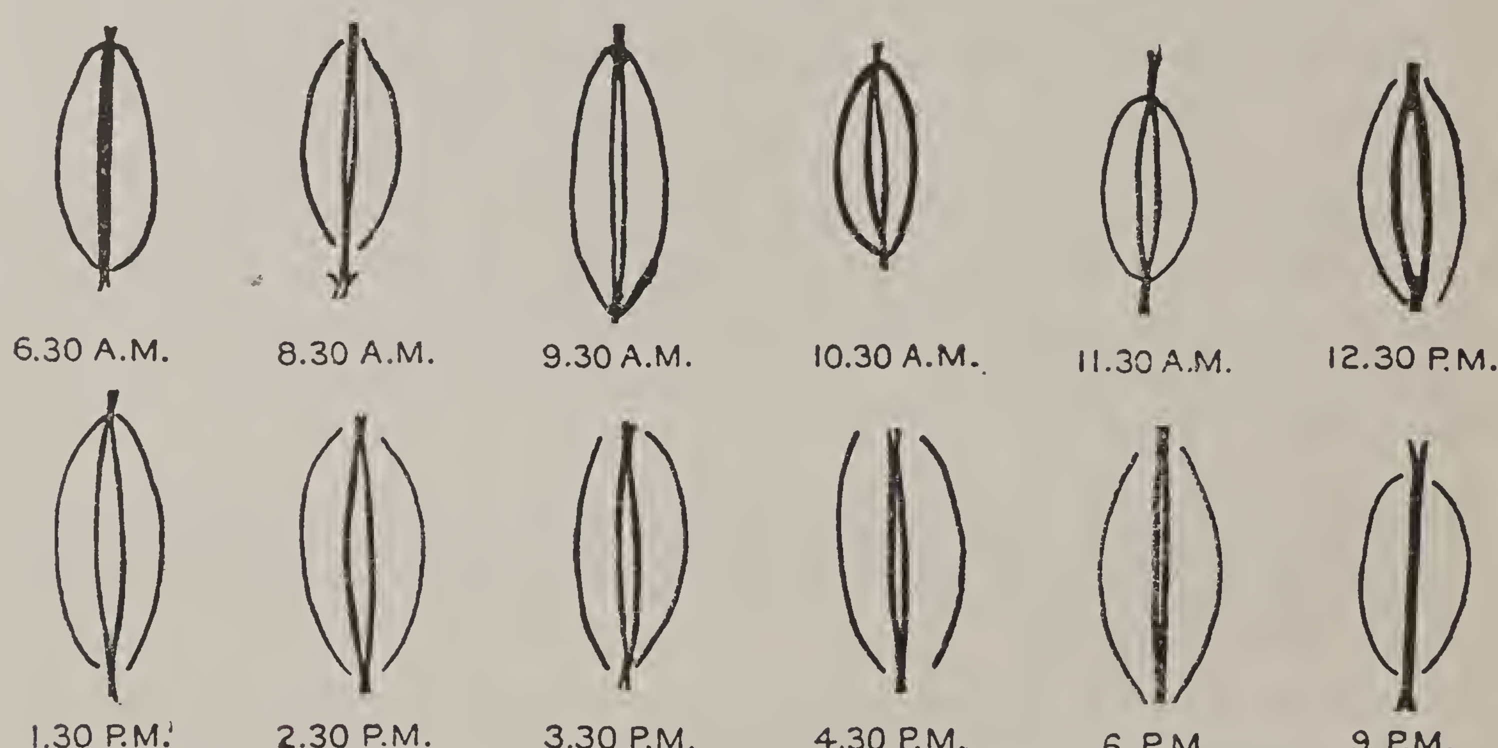

Fig. 1.—Diagrams of stomatal slits of the Monterey pine during the first week in October 1924.

The temperatures near the Coastal Laboratory stand above freezing-point, except for a few hours each year. The amplitude of variation increases with the onsent of growth, reaching a maximum about April, when the rate of net enlargement of the trunk is highest. It decreases with slackening growth to a minimum in August in all trees, whether in a moist or dry location. The minimum is maintained until mid-winter, with occasional periods of wider amplitude lasting for a few days only. The period of minimum variation and inactivity is shortest in young trees.

This reduction of the daily variation would be coincident with the period of minimum transpiration if the behavior of this pine is to be taken as similar to that of other pines. Weaver and Mogensen, working at the University of Nebraska with *Pinus ponderosa*, *P. banksiana*,

and *P. murrayana*, found that the winter transpiration was not more than 1/55 to 1/251 as great in these and other conifers as in the autumnal condition.[1] The Monterey pine may be taken to be in the autumnal condition in the summer months following the cessation of growth and in the winter condition in November and December at Carmel, California. The stomata were found to be active as late as the first part of October in 1924; whether they close for a period of days or of a few weeks in the mid-winter months is not known. (See figure 1.)

The ratio of the duration of the period of increase of the diameter to the period of daily decrease varies with the season in such manner as to indicate that this is a matter of soil moisture, although, as will be shown below, some evidence of internal changes is available.

During the midwinter period or in the earliest part of the growing-season the daily contraction may not be evident before 10 a. m. and swelling may begin by 3 p. m., so that the trunk is in a condition of increasing diameter for 19 hours of the day. By the time the condition of most active growth has been reached, this daily cycle has changed progressively, so that contraction of the trunk is discernible a half hour after sunrise and swelling does not begin until sunset and continues for about 12 hours out of the 24.

No inclusive or extended observations of the stomatal cycle have been made, but Dr. F. T. MacLean found that the stomata of this pine in July do not begin to widen until about 8 a. m.; that it continued until after $2^h\ 30^m$ p. m., but that the slit had narrowed to the morning dimensions by $6^h\ 30^m$ p. m. Observations by Mr. Magnus Gregersen in October gave similar results, which are illustrated by the camera drawings of the stomatal slits in figure 1.

The chief interest in this matter lies in the way in which such transpiration effects are transmitted to all parts of the trunk. This is much more striking in the case of the redwood (*Sequoia sempervirens*). One of these trees, 60 meters in height and 135 cm. in diameter at the base, was fitted with a dendrograph the floating frame of which was composed of fused silica rods, thus eliminating temperature errors. The lowermost branch is 22 meters from the base and the direct rays of the rising sun do not strike the crown until $6^h\ 45^m$ a. m. A contraction of the stem at the base has been repeatedly observed and recorded before 7 a. m. An examination of the stomata in July showed that the slits had begun to open before 8 a. m., were at their widest about 10 a. m., and had closed by 2 p. m. The onset of contraction and the closing are both more gradual than in the Monterey pine. The altered tensions in the water column in this case are transmitted a distance of at least 22 meters downward to the base of the stem where they may be recorded as alterations in diameter by the dendrograph.

[1] Weaver, J. E., and A. Mogensen. Relative transpiration of coniferous and broad-leaved trees in autumn and winter. Botan. Gaz., 68, 393–424. 1919.

The average length of the tracheary element in this *Sequoia* is 6.5 mm., according to Professor Bailey,[1] and the perforations in the pit-membranes are sufficiently large to permit the passage of the particles of carbon in india ink. The complex column of ascending water would therefore pass an average of nearly 3,400 tracheids. The resistance would, of course, vary with the rate, but a practical estimate is to the effect that the pull which would carry solutions to the summit of this tree would be equivalent to that of the weight of a column of water twice the height of the tree, or 120 meters.

The regulatory action of the stomata has been seen to be one of the main factors in the determination of the daily course of transpiration in a wide variety of types of shoot, including the succulents. Huber has recently described a similar dominance of this condition in the transpiration of the giant *Sequoia*.[2] Such a relation is evident in the Monterey pine.

The Monterey pine, unless growing in a very close stand, retains its lower branches, so that the total transpiratory pull or tension would be exerted only in the basal section of the trunk, the portion usually under measurement by a dendrograph. Solutions entering the roots would pass about 5,000 tracheids in reaching the terminals.

THE COURSE OF THE REVERSIBLE VARIATIONS IN THE TRUNK AND ROOT OF A SINGLE TREE.

Dendrographic records of the basal part of the trunk of Monterey pine No. 1, from September 1918, and of a region 8 meters higher from January 1920 to date and measurements of the growth and variations of a large root of this tree, include information by which a fairly complete scheme of the changes in volume of the entire trunk may be made.

The general features of the reversible variations of the trunk have been described in the preceding section. The trunk of the tree may be said to be in a state of enlargement during the period in which the stomatal slits are narrowest and transpiration is least. This takes place chiefly at night, during periods of high relative humidity, and under certain other exceptional conditions. Contraction of the trunk usually begins shortly after dawn and continues during the period of high transpiration, including that of the widely opening stomata. The departures from the general program of reversible changes in the trunk will be discussed in connection with the variations in roots which accompany them.

The measurements of the variations in roots were begun in January 1923. A dendrographic lever set on a suitable base was firmly fixed

[1] Bailey, I. W., and W. W. Tupper. Size variation in tracheary cells. Proc. Amer. Acad. Arts and Sciences, 54, No. 2. 1918.

[2] Huber, B. Transpiration in verschiedener Stammhöhe. I: *Sequoia gigantea*. Zeitschrift f. Botan., 15, 30. 1923.

on the concrete floor of a small chamber which had been made to uncover a root 2 meters from the base of the trunk, where it was 75 mm. in thickness. The horizontal sliding rod of fused silica was given a bearing on the surface of the root, which was so firmly embedded as to need no support. The covering provided for the chamber closed it so tightly that the air around the root was high in moisture, so much so that the record was blurred in places. (See figure 2.)

FIG. 2.—Arrangement of dendrograph in underground chamber to measure and record variations in diameter of a large root. Temperatures were taken by a thermometer inserted in the root.

It was thought remarkable that growth of this part of the root did not begin until the mid-season of the trunk had been reached, which was mid-April in 1923. It is well known that the apical growth of roots of this pine begin at a much earlier date, and it is highly probable that the rootlets of this one had been active for some weeks. Previous to the beginning of growth in the root, in March 1923, some daily reversible variations, the first of the season, became apparent. At this time enlargement began between 6 and 8 a. m. and continued until evening in reverse of the daily fluctuations of the trunk of the tree. Actual enlargement began a month later and continued for a month, with a total accretion of about 1 mm. in diameter.

This daily cycle became belated, so that about the first week in June enlargement did not begin until after midday. By the end of this month the beginning of enlargement was advanced so that it began early in the morning, as earlier in the season.

Such a daily program continued until the fluctuation lessened to a minimum about the first of September. Occasional variations were discernible in the records which were kept until mid-October 1923.

The instrument was again put in place on May 19, 1924, at which time growth for the season had ceased in the trunk and also in the root. The daily variations in the root were of the same type as at the corresponding time of the previous year. Enlargement began 2 or 3 hours after midday and continued until midnight. Fortunately, the installation had been made in time to record an advancement which took place late in June 1923. In this, the drier season of 1924, in which growth of trunks had ceased by May 1, the advancement of the daily cycle in the root was so abrupt that enlargement which began at 2 p. m. on May 31, was seen to set in at 8 a. m. on June 2. The onset was advanced to 6 a. m. by mid-June and on a few mornings was seen to occur as much as half an hour earlier and nearer sunrise.

Returning now to the variations in the trunk, it is to be noted that the onset of the swelling advanced in the trunk with the progress of the season, but more markedly so in the basal part than in the section above.

Late in May the increase in the basal section might begin as early as 2 p. m. or 5 hours earlier than the increase in the upper part of the stem. This relation was accentuated with the march of the dry season, as noted below.

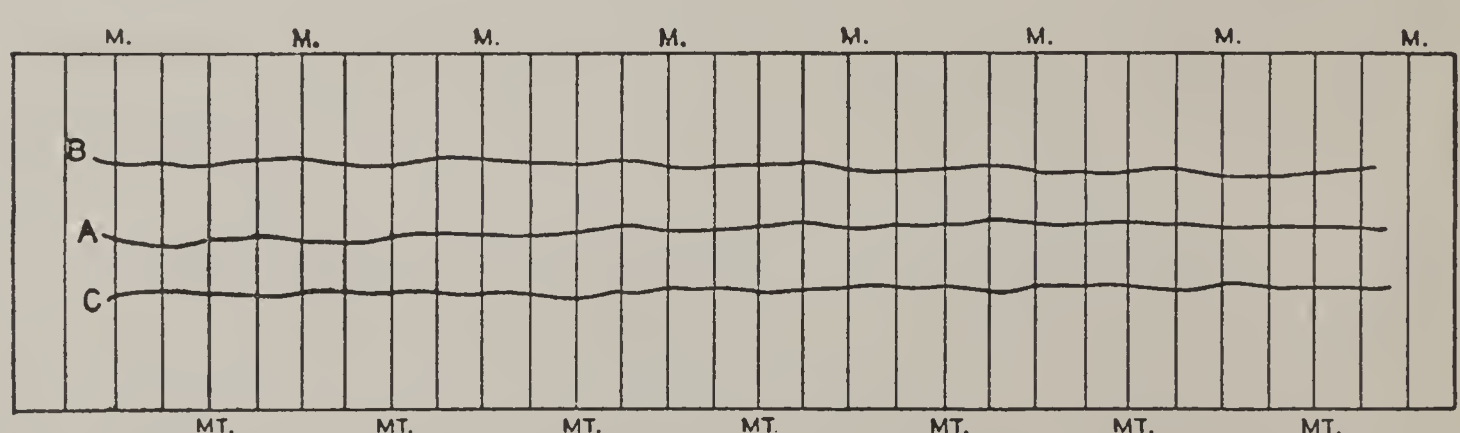

Fig. 3.—Facsimile of dendrographic records of a large root, the basal and the upper part of the trunk of Monterey pine No. 1, during the week beginning June 9, when the daily variations showed a departure from the usual procedure. This aberration consisted in a delayed expansion of the root and an advanced swelling of the base of the trunk. *A*, record of basal part of trunk, ×10; *B*, record of upper part of trunk, ×10; *C*, record of root, ×20.

It is thus seen that the cycle was changed so that the two phases in the root were at first directly opposed to those of the stem or trunk, then became belated during a month by a displacement of 6 to 8 hours, then advanced to the original opposition to the trunk. For example, on June 4 the increase in the root began at 8 a. m., of the

basal part of the trunk at 1 p. m., and of the upper part of the trunk at 7 p. m. The extreme expression of this change was found on June 12, 13, and 14, when the root began to increase at 6^h 30^m a. m., the basal part of the stem at 10 a. m. and the upper part of the stem not until after 8 p. m. The last week in June was marked by a still further advance of the beginning of increase of the root which took place at dawn, soon after 4 a. m. (See fig. 3.)

The soil to a depth of 0.5 meter about the base of this tree showed a moisture content of less than 4 per cent on July 1, at which time the above extraordinary relative action of the root, the basal and upper parts of the stem, was prevalent.

The tree was irrigated by running several thousand liters of water into holes at a short distance from the trunk, beginning on the evening of July 3; 48 hours later very little change had taken place in the time of the variations. On the third day the onset of the swelling action was advanced 2 hours in both places in the trunk. In the following week, which was characterized by high fogs or clouds, the onset of swelling in the trunk advanced to midday, beginning at noon in the basal part of the trunk on two days.

Another feature to be considered is that of the duration of the period during which the transpirational pull lessens the cross-section of the conducting elements and the trunk contracts, or, for convenience, the period in which the transpirational pull begins to lessen and the tracheary elements to expand, probably accompanied by a direct hydration and dehydration of the living cells when the full trunk is measured.

This may be expressed conveniently by measuring the total length of the dendrographic tracing in which such enlargement is recorded. These are given in millimeters in table 1, and it is to be noted that 40 mm. is equivalent to 24 hours, and that the record for the 6 days estimated would be expressed as 240 mm.

Table 1.

Date of beginning of week.	Root.	Basal region.	Upper part of trunk.
1924.			
Jan. 14	....	175	168
Mar. 24	....	190	195
Apr. 21	....	185	187
May 26	80	150	135
June 9	90	180	125
June 23	85	170	150
June 30	108	197	182
July 7	122	190	170

The duration of swelling or of increase is about the same in the basal and upper parts of the trunk during the growing period, the totals for January, March, and April given above being identical.

After the cessation of growth, with many warm days and a soil with decreasing moisture content, the totals for three regions were 500 for the basal part of the trunk, 410 for the upper part of the trunk, and 255 for the root, which showed no definite change in this relation. Thus the figures for the root in successive weeks ending with that beginning June 23, as given above, are 90, 80, 95, 90, 90, 85.

The pronounced and definite increase in the length of time during which the upper part of the trunk was in course of enlargement in the last week in June took place at a time when the supply of soil moisture was at a minimum, the time of swelling of the base was at a minimum, and that of the root was decreasing.

It is to be noted that water, in passing from the region of the root in which variation was measured, would pass through but 2.2 meters of tracheary conduits in reaching the diameters of the trunk which were being recorded by a dendrograph. What transformation or modification of cohesion tension might take place in this basal region of the trunk and root is not easily to be described.

On the evening of July 3, when the tree was in a condition that the enlargement of the root began at 6 a. m. or earlier, of the basal part of the trunk at 2 to 4 p. m., and of the upper part of the trunk at 6 p. m., the soil, which now had a minimum proportion of moisture, was saturated with water by the application of about 14,000 liters in large holes a short distance away from the base. The results were striking and changes were visible within a few hours. The large root showed a contraction on the following day almost exactly in reverse of its usual procedure; contraction began at 5 a. m. and continued until 7 p. m., when enlargement began and continued until 2 p. m. The subsequent contraction continued until the following morning, when the cycle was resumed of swelling in the daytime and contraction at night, with the accompaniment of some permanent enlargement of growth. (Fig. 4.)

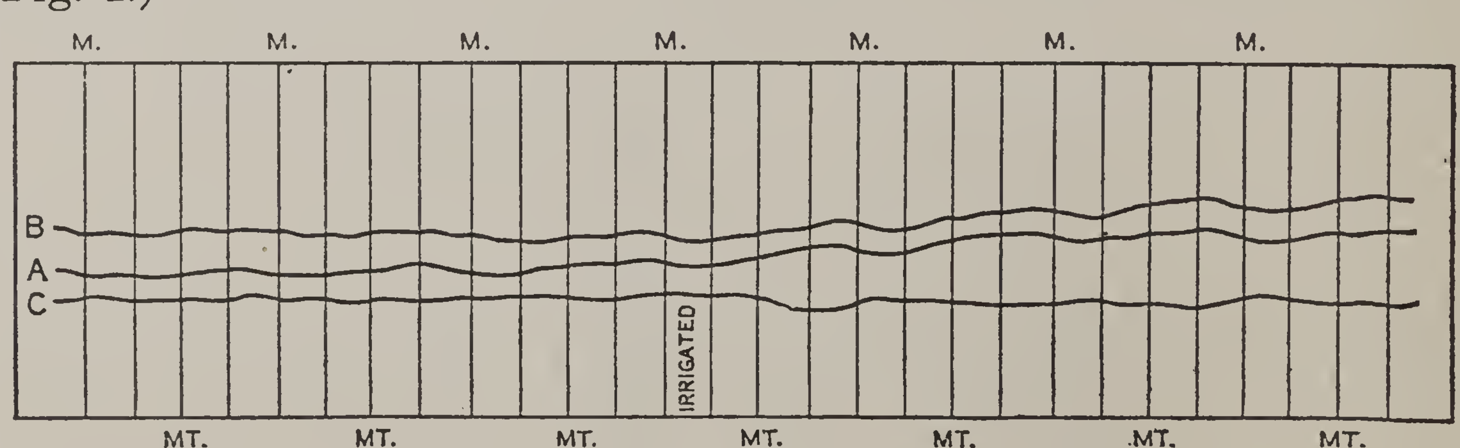

Fig. 4.—Record of reversible variations of trunk and root during the week beginning June 30, in which growth began anew as a result of irrigation. *A*, record of basal part of trunk, ×10; *B*, record of upper part of trunk, ×10; *C*, record of root, ×20.

The night following, irrigation was accompanied by an expansion of the trunk both below and above, which continued until 9 a. m. in the upper part of the trunk, the subsequent contraction ending at

3 p. m. The swelling of the basal part of the trunk continued until about 9 a. m., then no positive change was visible until 2 p. m. when contraction for 3 hours followed. Enlargement began before 6 p. m. and the usual alternation of daylight contraction and nocturnal swelling was resumed. The amplitude of the variations was small, as in the beginning of the growing-period early in the year. The trunk now took up a second period of enlargement, as when irrigated at the corresponding period in 1920 it also displayed some growth activity. The duration of its swelling periods remained undiminished, while these periods were shorter in the trunk during the second week following irrigation. (Fig. 5.)

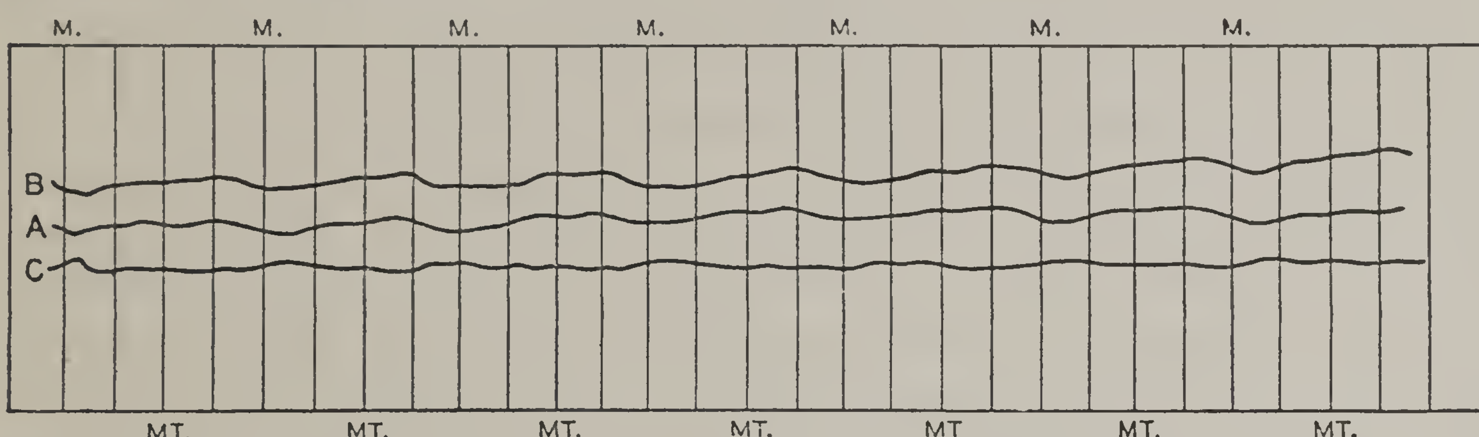

Fig. 5.—Record of variation in the trunk and root of the Monterey pine for the week beginning July 28, 1924. *A*, record of base of trunk, ×10; *B*, record of upper part of trunk, ×10; *C*, record of root, ×20. Second growth-period of season following irrigation.

It is apparent that the tree does not present a single unicyclic arrangement of variations in cohesion tension consequent upon or accompanying periods of increased and lessened transpiration. Thus, in the mid-season of the growth of the trunk and previous to the onset of enlargement in a large root, this root showed reversible variations exactly opposed to those of the trunk. Enlargement took place during the daytime and contraction at night. The enlargement then became belated, so that it did not begin until after midday during 1924.

The same root in 1924 displayed an enlargement after midday in May as noted above, continuing the increase until midnight. With the advance of the season, the onset of enlargement was abruptly advanced so that on May 28 the increase began in the early morning, and in June it began before sunrise, at a time when the soil-moisture content was extremely low.

Late in May the hour of enlargement of the base of the trunk was also advanced 2 to 5 hours, and a still further advance parallel to that of the root followed, so that in mid-June the base of the stem began to enlarge by 10 a m. The trunk at a distance of 8 meters from the base continued its regular cycle, always beginning to contract before 8 a. m. This sudden advance of the cycle in the root and base of the stem was also characterized by the fact that the length of time over which the enlargement continued in these regions was lengthened, as shown

in the record for the week beginning June 9, figure 3. A similar lengthening of the daily swelling period resulted from its continuance later in the morning hours.

The measurements given above establish the fact that the root undergoes a daily fluctuation in diameter which may amount to as much as 1 part in 750 of its diameter. This variation is most marked in the period in which the trunk and this heavier part of the root are in a state of active enlargement. It is also seen previously to the beginning of growth in the larger part of the root. As these variations do not coincide with the phases of variation in the trunk, the correlation between the two is not direct.

The time and the rate at which the diurnal fluctuations take place may be taken to give some clues as to their nature. These reversible changes were measured in the older part of the root and were seen to begin when the trunk had been in a condition of active growth for some weeks and the tips of the rootlets had also been active, perhaps, for even a longer period. The seasonal beginning of the reversible variations was, however, at a time when the part of the large root under measurement had not begun to enlarge. As the phases of the fluctuation are opposed to those of the trunk, the direct action of transpiration in the crown of the tree acting on the water column of the root can not be given as the cause of such variations. (Fig. 6.)

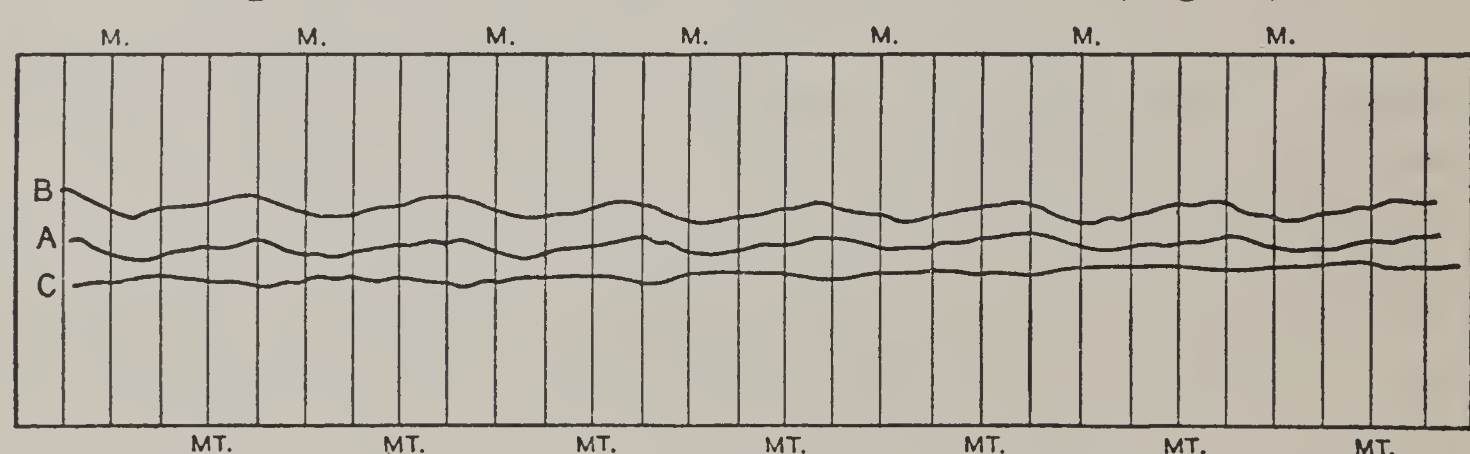

Fig. 6.—Record of variation in root and trunk of Monterey pine for the week beginning September 22, 1924, in second growth-period of season following irrigation. *A*, record of basal part of stem, ×10; *B*, record of upper part of stem, ×10; *C*, record of root, ×20.

The gradual onset and advance of the enlargement of the large root would indicate that it is to be ascribed to changes in turgidity of living cells in the cortex of the root rather than to changes in cohesion tension of a water column in xylem, or non-living tracheids. A thermometer inserted in the large root near the contacts of the dendrograph showed variations between 17° C. at 8 a. m. and 20° C. after midday. The conditions would correspond to those in the soil at a depth of about 15 to 20 cm. on level ground. The terminals of the rootlets would lie at about this depth and would be subject to changes of about the same character and amplitude. Rising temperature would increase osmotic action in the absorption of water across the endodermis in the young root-tips, send an increased supply toward the larger

main root, which, also affected by the temperature, would show an increased osmotic action resulting in a higher degree of turgidity of the living parenchymatous cells.

That the variations in the osmotic action of the contents of the living cells of the root would be sufficient to cause changes in turgidity which would be expressed in altered diameters of the roots becomes evident when it is considered that the osmotic pressure of a 0.1 normal solution of cane sugar is 0.05 atmospheres greater at 20° than at 15° C.

That the reversible variation is in this case determined or affected by the temperature of the soil in which the roots are embedded is in contradiction to the conclusion reached by authors who have measured root-pressures or bleeding-pressures by calibration of the amount and pressure of bleeding.[1]

A conclusion that such exudation pressures, which are dependent on osmotic action, should be independent of temperatures is so remarkable that it deserves a reinvestigation. It is highly probable, however, that the course of an exudation pressure released by decapitation or wounding is such as to mask and conceal a temperature effect.

That the fluctuations may be determined chiefly by other conditions or factors is a possibility. Preliminary measurements of the changes in volume of a root of *Sequoia sempervirens* less than a meter from the base of the trunk reveal the fact that the phases in this case are approximately identical with those of the trunk, contraction ensuing during the day and expansion at night. This behavior suggests that the tension set up by the transpiratory action of the leaves may extend downward into the root in this tree, a matter which will be discussed in the pages that follow.[2]

The following features are to be taken into account in any attempt to correlate changes in volume of root and trunk of the Monterey pine: (Fig. 7.)

1. The onset of enlargement of the root in a high soil-moisture takes place in early morning before contraction of the stem has begun, near the end of a nocturnal period of low transpiration.
2. The close of the growing-season with minimum soil moisture is characterized by a delayed daily enlargement of the root. Instead of preceding the daily contraction of the base of the trunk the expansion of the root does not take place until midday or later after contraction of the trunk has been in progress for several hours. This occurred in June 1923 and in May 1924 in which growth ceased earlier in the season than in the preceding year.
3. Delayed daily enlargement of the root covered a period of a few days, in which the onset of enlargement of the base of the trunk was advanced to midday from about sunset.

[1] Palladin, V. I. Plant physiology. Ed. by B. E. Livingston, p. 128. 1917.

[2] For a discussion of the mechanism of absorption and root-pressure see:

Priestly, J. H. The Mechanism of root-pressure. The New Phytologist, 19, 189–200. 1920.—Further observations upon the mechanism of root-pressure. The New Phytologist, 21, 41–47. 1922.

Blackmann, V. H. Osmotic pressure, root-pressure, and exudation. The New Phytologist, 20, 106–115. 1921.

4. The change from this displaced position of both of these points was abrupt. The onset of swelling of the root was before 2 p. m. on June 1, 1924. On June 3 it had advanced to 8 a. m. The onset of the swelling of the basal part of the stem was at 2 p. m. on the 20th. It had taken its normal place on the 26th; the record of the 23d, 24th, and 25th were faulty, but it appeared that the hour of beginning was moving gradually toward 6 p. m., which was shown on the 26th. This took place a week before the root had resumed its usual, or normal, cycle.

5. The disturbance took place at the end of the growing-season in a period of minimum soil moisture and ended with the soil moisture still low.

6. Irrigation, restoring the high soil-moisture content of the soil, resulted in lengthening the swelling period of the trunk notably, that of the root less.

7. The onset of swelling of the root now began before the close of the enlargement of the trunk. Enlargement continued through the contracting period of the trunk and closed after the trunk had been in a state of enlargement for a brief period, perhaps no more than an hour at the base.

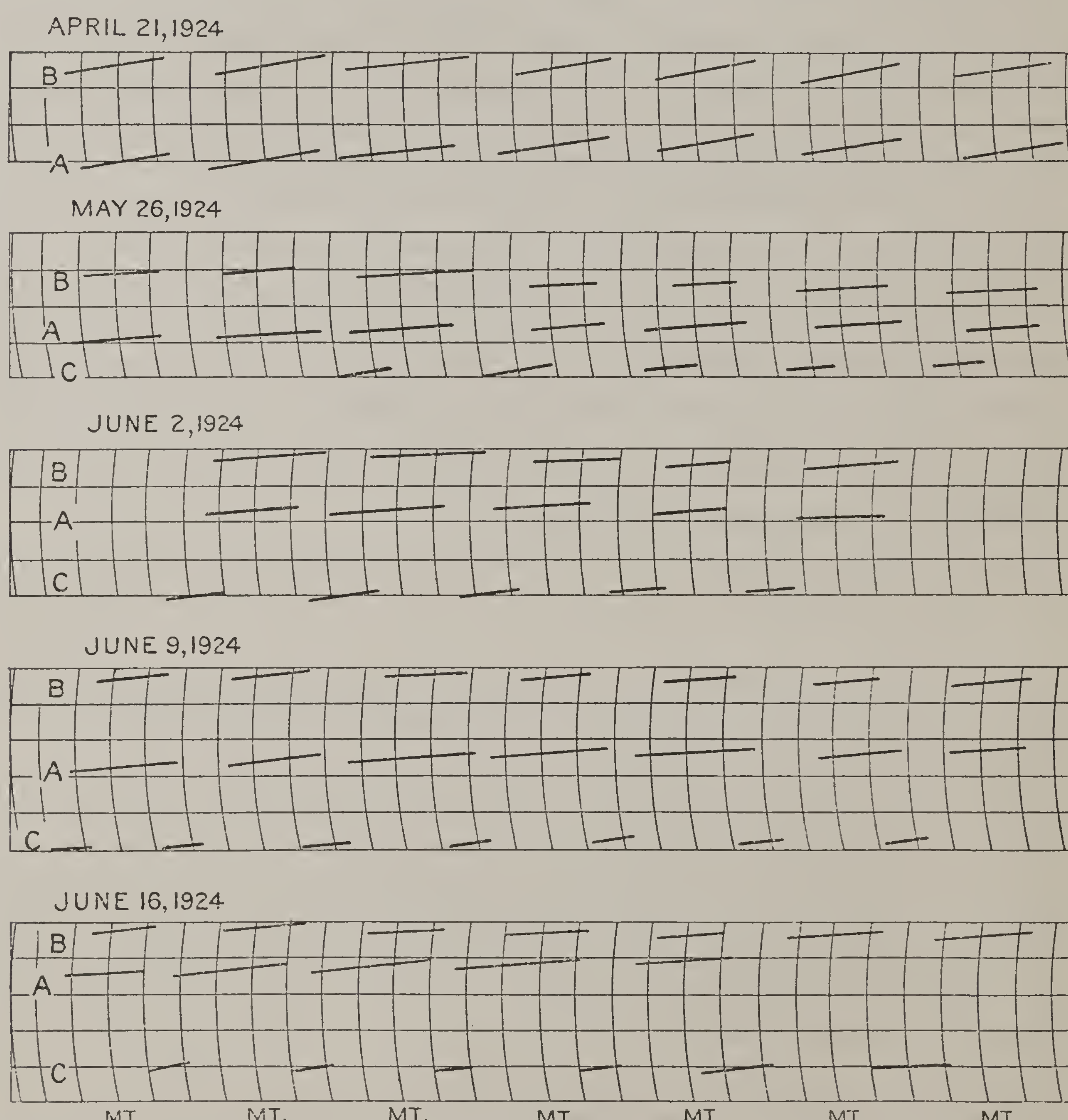

Fig. 7.—Diagram showing relative periods of expansion of root (*C*), basal part of the trunk, (*A*), and upper part of the trunk (*B*) of the Monterey pine during 5 weeks in 1924. A departure from the usual procedure was shown in the week beginning June 9.

It is to be admitted that complete and adequate explanation of the disturbances summarized above can not be made on the basis of present information. It is equally certain that other important agencies besides temperature have participated in determining the phases of contraction and expansion.

The delayed expansion of the root during the morning hours with a rising temperature might be ascribed to the slow acquisition of water necessary for increased turgidity. That, however, under conditions of increasing desiccation, swelling should again begin early in the morning, completely invalidates such an attempt at explanation.

It will be necessary to seek the causes of these disturbances in the behavior of the stomata and the condition of the tension in the column of water during the period in question. Unfortunately, no observations on the stomata were made during this time, so that nothing can be said as to possible departures from the daily program by which the slits begin to widen in the morning and close at midday, reaching the narrowest position in mid-afternoon.

If the premature onset of swelling of the basal part of the trunk were due to earlier closure of the stomata, all parts of the trunk would be affected in an identical manner. It is only the basal portion, however, in which premature swelling occurs. The disturbance of the diurnal program was in the basal part of the trunk and the large roots within 2 meters of the base of the trunk. There are two other conditions which might be concerned. One would be the complex water column in the wood and the other the neutral zone which must exist in the region where the cohesion tension is met by the head of root-pressure.

If the transpiring stream be conceived as a complex column filling the network or layers of tracheids, it is conceivable that with an increased tension the branches of the column might become broken in certain layers, and then the pressure column from the root rising below might push a head of water up into the basal part of the stem, with a resultant increase in size of the tracheids by a repletion with the water. Whether or not air-bubbles which would be pulled in the tracheids would be pushed out by the rising head is doubtful. Furthermore, it is not seen that the implied conditions would account for the displacement of the phases of increase and contraction in the root. In any case, the water column under tension would persist, although it might not occupy so large a tract of the wood. Holle found that intact water columns were to be seen in the vessels of plants the leaves of which had completely wilted,[1] and my own observations of the reversible variations in the dead trunks of trees support the inference that even here a continuous column exists which extends from the leaves downward.

[1] Holle, H. Untersuchungen uber Welken, Vertrocken and Widerstraffwerden. Flora, 108, 73. 1915.

Still another supposition would be that the disturbances in the basal part of the stem would set in action the series of osmoses usually expressed in exudation pressure, and these might cause the changes in volume which have been noted. Such a behavior was recorded but once in 6 years' continuous record of this tree, and it was shown at a time when the lowest soil-moisture record at the Coastal Laboratory had been made, the water-content of the soil being less than 4 per cent.

Still another aspect of the unusual variations in the trunks is suggested by a discussion of relative vapor-pressures in the air, in the menisci of the transpiring cell-walls, in the vacuoles of the transpiring cells, and in the tracheids, by Professor Dixon,[1] who points out that when the menisci are drawn deeply into the interstices of the walls the vapor-pressure will be so reduced that it may not be as great as that of the surrounding air. In this condition the upward movement of water in the column under tension would stop, and this may go so far that water would be condensed on the surface of the walls and the movement in the tracheids might be downward. It is not clear, however, that such action could be held to account for a change in the reversible variations in the basal part of the trunk without affecting the action of the length above.

ROOT-PRESSURE AND EXUDATION PRESSURE.

The secretory action by which living cells take in water endosmotically and force it through walls into non-living xylem elements or into cavities or on to surfaces is one which is exhibited in a variety of arrangements in the plant. Much of the present confusion regarding this subject may be ascribed to the custom of regarding all positive excretory action of bleeding of stems to such osmotic action in the root. The intake of water across the endodermal membrane of the terminal parts of growing roots doubtless does result in filling the xylem in these members and with such pressure as to force the liquid upward in the vessels, as far as the leaves in herbaceous plants and other small shoots. The positive pressures which may be registered by gages sealed over the stumps of such plants may be in actuality set up in the roots. It might be safely inferred in such instances that the entire basal part of the shoot in such case was filled, perhaps completely, with a complex water-column.

On the other hand, no positive evidence can be found that the head of water raised in the xylem by endodermal action actually finds its way very high in the base of the trunk of a Monterey pine, unless the facts described on the preceding pages constitute such an occurrence.

The parenchymatous cells of the xylem, the ray-cells which are in contact with the wood-cells of the ray and with the vertical or

[1] Dixon, H. H. The transpiration stream. University of London Press. 1924.

axially placed tracheids, constitute a mechanism much like that of the root. They may absorb water endosmotically and excrete it into cavities or non-living cells contiguous to them with a pressure which may be measured by manometers. The best arrangement is one in which a cavity would be made in the layers of wood with a continuous water column in which the ray-cells were alive and turgid and which might take in water from wood-cells and excrete it through the exposed walls. It can not be stated with assurance that these living cells maintain a constant exudation action to the axially placed wood-cells, although many writers attempt to maintain that by such pumping action sap is driven upward in trees. It is to be kept clearly in mind, however, that the pressure recorded by a gage affixed to a cavity in one part of the trunk of a tree might have but little connection with that in another place. The pressure set up in any case would be a direct result of the action of the living cells affected. The osmotically active contents of the parenchyma vary widely in different parts of the tree.

The variations in exudations might have been expected to furnish a basis for their analysis, but so far their irregularity has baffled all investigators, beginning with Hofmeister[1] in 1862, who first observed them, and later Baranetzky,[2] who made them the subject of systematic measurement.

EXUDATION PRESSURE IN THE MONTEREY PINE.

Conclusions as to the localization of the activities giving rise to exudation pressures and the improved technique by which this may be demonstrated are discussed below. As will be shown, the measurements made from stumps and entire cross-sections of stems are wholly inadequate in the pines and are subject to serious criticism in all plants. The thick layers of wood formed annually by the Monterey pine makes it possible to calibrate the action in the layer of any one or two years. While the pressures measured were much above any available or obtained by other workers, yet it is by no means asserted that maximum exudation pressures are shown by the pines. The application of improved methods would doubtless demonstrate still higher maxima in other trees or shrubs which are known to bleed copiously.

Several experiments were carried out for the purpose of testing exudation pressure which might be set up in the basal parts of trunks of the Monterey pine. The first one was with a small tree which was still growing and had a trunk 10 cm. in diameter a meter above the base. A metal tube was screwed into a shallow hole bored a few

[1] Hofmeister, W. Ueber Spannung, Ausflussmenge und Ausflussgeschwindigkeit von Säften lebender Pflanzen. Flora, 45. 1862. A series running in 5 numbers of the volume.

[2] Baranetzky, J. Untersuchungen ueber die Periodicität des Blutens der Krautigen Pflanzen und deren Ursachen. Abh. Naturf. Ges, z. Halle, 13. Halle. 1873.

centimeters into the trunk a meter from the base, mid-June 1924, at a time when the soil moisture was at the minimum indicated. A suitable system of tubes with a water-supply and mercury column was attached. A pressure of 112 mm. of Hg was developed on the second day of the test, which slowly fell on the fifth day to 80 mm.

A pressure apparatus consisting of a long U tube joined by a rubber tubing to a metal tube was attached to the basal part of the trunk of Monterey pine No. 2, on which dendrographic measurements had been made in previous years. A filling funnel with stopcock was included in the system between the U and the metal tube. The apparatus was put in place June 16, 1924. So much resin poured into the cavity, which had been bored out to a depth of 5 cm., that the pressure was sealed 6 hours after beginning at 27 mm. Hg. The apparatus was disconnected on the following day, the resin cleaned out, and the mercury column set at zero. On the following morning, 20 hours after being set in action a second time, the pressure was 158 mm. Hg; 24 hours later it had risen to 181 mm. Hg, where it remained stationary for one day, then began to fall, so that two days later the reading was 140 mm. Hg. This is in accordance with the general course of exudation pressure measured with the U-tube manometer.

The exudation of sufficient liquid to fill the column under these mounting pressures is of course to be considered as a complex result in which variations in the flow are caused by changes in the pressure against which the flow operates.[1]

No variations in the pressure which could be correlated with the temperature of the living cells which are the seat of the forces which cause exudation could be detected by the crude method of measuring the height of the mercury column from time to time. It is highly probable that the osmotic action of these cells would show a temperature effect.

No relation of the changes in the pressure to the daily reversible variations in the trunk as recorded by the dendrograph could be detected. The method of measuring the mercury column would be inadequate to determine such a relation.

Later, in August, a similar apparatus was attached to the basal part of Monterey pine No. 1. The U tubes filled with mercury were attached to a hole bored in the basal part of the trunk, which had been under dendrographic measurement since September 1918. A second dendrograph has also been attached to the upper part of the trunk for 4 years, and an instrument had been arranged to record variations in a large root in March.

The system was sealed into the trunk at 4 p. m. on August 26, the attachment being made by a brass tube screwed into the outer end of

[1] Chamberlain, H. S. La Sève Ascendante. Bull. d. Lab. d. Botanique, 2, 1–340. Neuchatel, 1897. Cited by J. H. Priestley, New Phytologist, 21, 44. 1922.

a hole about 5 cm. in depth which had been bored into the tree, the inner end of the cavity being lower than the opening. The night and morning following were foggy. At 8 a. m. on the 27th a pressure of 332 mm. Hg had developed, and, as may be seen from the figures below, rose rapidly. Pressures are recorded in table 2.

TABLE 2.

Date.	Time.	Tree temp.	Air temp.	Pressure in mm. Hg.	Remarks.
		° C.	° C.		
Aug. 26	4 p. m.	Set up.			
Aug. 27	8 a. m.	12.5	....	334	Foggy.
	9 a. m.	....	....	344	Do.
	10 a. m.	13.5	14	384	Do.
	10h 45m a. m.	14	14	404	Do.
	11 05 a. m.	14.5	15	410	Sun coming out.
	11 30 a. m.	14.5	15	420	
	12 noon	15	15	430	
	2h 10m p. m.	15	15.5	470	
	3 30 p. m.	16	16	480	
	4 30 p. m.	15	15	485	
	5 30 p. m.	14	14	500	
	6 p. m.	13	13	505	
Aug. 28	8 a. m.	12	12	530	

The pressure had reached the limit of the U tube, water being forced past the bottom of the mercury column. The tube screwed into the trunk and the connecting T were becoming clogged with resin. The apparatus was accordingly dismounted and the U tube with an open end was replaced with a manometer in which the column of air in the closed arm which would be compressed by rise in the pressure was 136 mm. in length. The replacement was made 41 hours after the original attachment was made, and the pressure was set at zero with the new fitting. The results obtained are given in table 3.

TABLE 3.

Date.	Time.	Tree temp.	Air temp.	Length of air column.	Remarks.
		° C.	° C.	mm.	
Aug. 28	9 a. m.	13	13	136	Sun coming out; contraction of trunk began at 10 a. m.
	12 noon	15	16.5	133	Sunshine.
	2 p. m.	15.5	16.5	135	Overcast.
	4h 30m p. m.	15.5	15	133	Do.
Aug. 29	8 a. m.	12.5	12.5	136	Foggy; no pressure; contraction of trunk began at 10 a. m.
	11h 30m a. m.	16	14.5	135	+1 mm.
	4 p. m.	16.5	16.5	135	+1 mm.; overcast.
Aug. 30	8 a. m.	12.5	12.5	136	+0; overcast.

The apparatus was now taken down and the tube found to be filled with resin. The brass tube was now screwed to the depth of 1 cm. into another hole on opposite side of tree, and lower down, at 9 a. m. At noon a "negative" pressure of 3 mm. was developed. The tubes and couplings were refitted at 3 p. m., as at that time the pressure had come back to zero. Readings as given in table 4 were made in the four days following.

TABLE 4.

Date.	Time.	Tree temp.	Air. temp.	Length of column.	Remarks.
		° C.	° C.	mm.	
Aug. 30	3 p. m.	15.5	15.5	136	
	4h 30m p. m.	15.5	15.5	129	
Aug. 31	8 a. m.	12	12.5	56	Pressure, 2.43 atmospheres; overcast.
	10 a. m.	14.5	15	55	Fog lightening; contraction of trunk began at 9 a. m.
	11h 30m a. m.	15+	17+	54	Pressure, 2.5+ atmospheres.
	12 30 p. m.	16	17.5	53	Brighter, but no direct sunshine.
	5 p. m.	(1)	(1)	56	Pressure, 2.4 atmospheres.
Sept. 1	8 a. m.	15	14	50	Pressure, 2.72 atmospheres.
	9h 30m a. m.	16.5	15+	50	Overcast.
	10 30 a. m.	17	16	51	Sunshine.
	11 a. m.	21	17	51	Do.
	2 p. m.	20	18	52	Do.
	5 p. m.	20	20	52	Do.
	7 p. m.	15	16	51	Do.
Sept. 2	8 a. m.	14	15	51	Do.
	9 a. m.	17	20	51	Do.
	11 a. m.	20	22	52	Do.
	4h 30m p. m.	17	18	53	Do.
Sept. 3	8 a. m.	13	13	53	Overcast.

[1] Warmer, but thermometers not read.

The apparatus was now dismounted and the pipe which was screwed into the tree found to be clogged with resinous material.

On September 1, at 2^h 45^m p. m., a closed manometer was connected with a hole in the trunk of Monterey pine No. 1, at a height of 8 meters from the base and near the dendrograph which had been in operation near that level since January 1920. The hole was bored cleanly with a sharp auger-bit in such manner that the inner end was slightly lower than the opening, and terminating in the wood of 1922, being driven tangentially. A threaded brass tube with a bore of 1 cm. was screwed into the opening, sealed with Canada balsam, and the hole filled with water within 5 minutes. The chips cleaned from the hole were moist and watery, and it was thought that the column of water in the wood was probably but little disturbed. The gage near the base of the trunk had that morning showed a pressure of 2.7 atmospheres, and at this hour it had fallen to about 2.5 atmospheres, partly by the error due to the expansion of the air in the manometer. The

temperature of the air was 20° C. and that of the trunk at the base was 18° C. The length of the air column was 74 mm. Additional readings are given in table 5. (Fig. 8.)

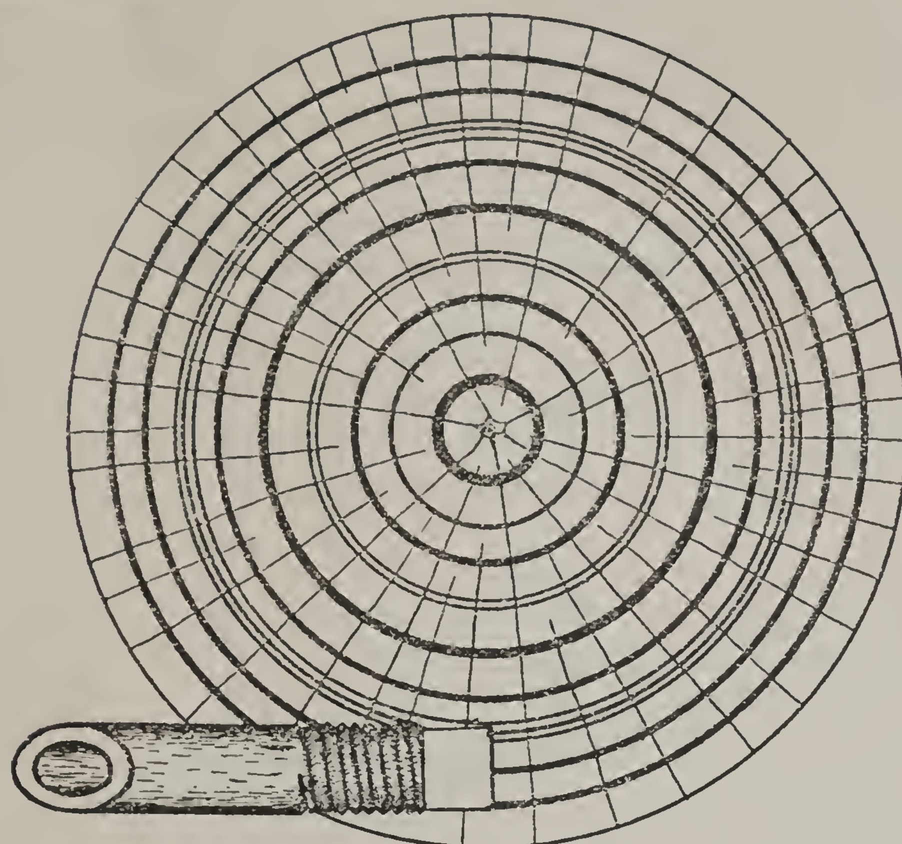

FIG. 8.—Diagram illustrating arrangement of tube screwed into outer layers of trunk of Monterey pine, to which is attached a manometer for measuring exudation pressure.

TABLE 5.

Date.	Time.	Length of column.	Remarks.
		mm.	
Sept. 1	3 p. m.........	90	Absorption by the wood had pulled column out to a "negative" pressure of 16 mm.
	4^h 30^m p. m....	90	Absorption satisfied.
	5 p. m.........	80	Pressure rising.
	7 p. m.........	78	Pressure rising; 4 below zero.
Sept. 2	8 a. m.........	28	Pressure 2.64 atm.; clear and warm.
	9 a. m.........	27	Pressure 2.7 atm.
	11 a. m.........	28	Pressure 2.6 atm.; air, 22° C.
	4^h 30^m p. m....	30	Pressure 2.46 atm.
Sept. 3	8 a. m.........	26	Pressure 2.85 atm.; air, 13° C.; tree, 13° C.
	8^h 45^m a. m....	25.5	Pressure 2.9 atm.
	10 30 a. m....	26	Pressure 2.8 atm.; air, 22° C.; tree, 19° C.
	2 45 p. m....	27	Pressure 2.74 atm.
Sept. 4	7 30 a. m....	27	Pressure 2.74 atm.
	11 a. m.........	27	Pressure 2.74 atm.
Sept. 5	8^h 30^m a. m....	28	

The gage was removed from the upper part of the trunk on September 8, the resin appearing to be liquid and not having choked the tubes. This material could not be reabsorbed, however, so that the pressure as noted might have been maintained indefinitely. The apparatus was now attached to the base of the trunk, at a point about 60 cm. above the base, for purposes of comparison with the action of the root, as shown by an instrument attached on the previous day.

Some delay was unavoidable in attaching the pressure-tubing and manometer to the brass tube after it had been screwed into the wood and water was absorbed which would have shown as negative pressure if the entire instrument had been put quickly and completely into operation. The hole was bored into the tree to terminate in the wood of the second year previous. Readings as given in table 6 were obtained:

TABLE 6.

Date.	Time.	Air temp.	Tree temp.	Length of column of air in manometer.	Remarks.
		° *C.*	° *C.*	*mm.*	
Sept. 8	$3^h 20^m$ p. m.	....	....	70	
	3 30 p. m.	....	....	69	
Sept. 9	8 a. m.	13	14	21	Pressure, 3.2 atm.
	$9^h 30^m$ a. m.	15	14	20	Pressure, 3.5 atm.
Sept. 10	7 a. m.	15	14	20	Pressure, 3.5 atm.
	9 a. m.	Tube co	nnection	ruptured.	

The above pressures are the highest recorded for any coniferous stem. The realization of these values may be ascribed to the technique by which a cavity bored into woody layers devoid of long vessels with a comparatively large mass of active parenchymatous cells tributary to the cavity, into which liquid is forced by exudation pressure, is connected directly with a manometer with a closed end. The necessity for such a method is determined by the nature of exudation pressures. It is agreed that the value of these pressures depends upon the osmotic action of turgid living cells with concentrated contents. These cells take up water and force it by exosmose through walls bounding the cavity into the liquid of the manometer tube, setting up a pressure that is registered at once. The concentration of the sap of the turgid cells is highest at the moment when the experiment begins. Some of the liquid in the manometer tube may be absorbed by the living cells or it may go into the wood cells, but within a few minutes the excretory action of the living cells adds to the volume of the water in the manometer, with the result that the air in the closed end is compressed. Obviously, the force with which such excretion takes place will be theoretically at a maximum in the beginning. Some amount must be excreted, however, in order to compress the column of air from its original to a lesser volume, registering the highest pressure. In the case described above the volume of the column of air was 0.35 c. c. at the beginning of the test. The cavity in the wood into which the manometer tube was screwed was 3 c. c. To set up the maximum pressure in the manometer used the air was compressed from 0.35 c. c. to 0.1 c. c., which may be ascribed to the excre-

tion into the liquid in the cavity of the trunk of 0.25 c. c. sap, which required 18 hours.

It seems highly probable that a manometer with a shorter arm or with a smaller bore would have registered this maximum earlier. The inadequacy of the older technique, by which open-arm manometers or U tubes were attached to stumps of stems and branches is illustrated by a test made on a small pine, 15 mm. in diameter, on September 10. The main stem was cut at a height of a meter and a manometer with a closed end was attached to the stump, which had a diameter of 6 mm.

While fitting a pressure-tubing to the stump it became apparent that water was being taken in, and after the manometer was attached a "negative" pressure was shown as given in table 7.

Table 7.

Date.	Time.	Length of column in manometer.	Remarks.
		mm.	
Sept. 10	10 a. m.	73	
	11 a. m.	78	− 5 mm.
	11^h 50^m a. m.	80	− 7 mm.
	4 p. m.	85	−12 mm.
Sept. 11	9 a. m.	83	−10 mm.
	11 a. m.	84	−11 mm.
Sept. 12	2 p. m.	78	− 5 mm.
Sept. 13	8 a. m.	76	− 3 mm.

The apparatus was dismounted and a large pressure-tube fitted over the end of a tree 22 mm. in diameter with 6 layers of wood, a meter from the base. The readings are given in table 8.

Table 8.

Date.	Time.	Length of column in manometer.	Remarks.
		mm.	
Sept. 13	3 p. m.	73	
	3^h 30^m p. m.	84	−11 mm.
Sept. 14	1 30 p. m.	80	− 7 mm.
	5 30 p. m.	80	− 7 mm.
Sept. 15	8 a. m.	77	− 4 mm.

This test also shows no positive exudation pressure.

Two days after the manometer had been affixed to the stump, another instrument was attached to the base of the stem by screwing a tube in a hole bored in the wood. No pressures were recorded, the stem having become inactive by the breaking of the column of water

at the top in affixing the first gage. The test was repeated. An instrument was attached to the base of the stem by screwing in a tube; a second instrument was attached to the branch which had become the leader. Within 5 minutes the gage attached to the stump showed a "negative" pressure. Ten minutes later the negative pressure was 8 mm. The instrument attached to the hole in the wood showed no negative pressure; on the contrary, a slight positive pressure was showing in the column, which was 50 mm. in length (above the water). 20 minutes after attachment to the stump a negative pressure of 11 mm. was shown, while pressure was making in the gage attached to a hole in the stem. On the following morning the negative pressure had been maintained at the stump, while the instrument attached to the side of the stem below showed a positive pressure which compressed the air in the closed end of the manometer from 50 to 48 mm. Higher positive pressures were developed on this and the succeeding day.

Another aspect of the difference between pressures registered on the cut ends of branches and those taken directly from the layers of wood was tested by fastening a manometer to the cut end of a branch of Monterey pine No. 1 at a distance of 8 meters from the base. The

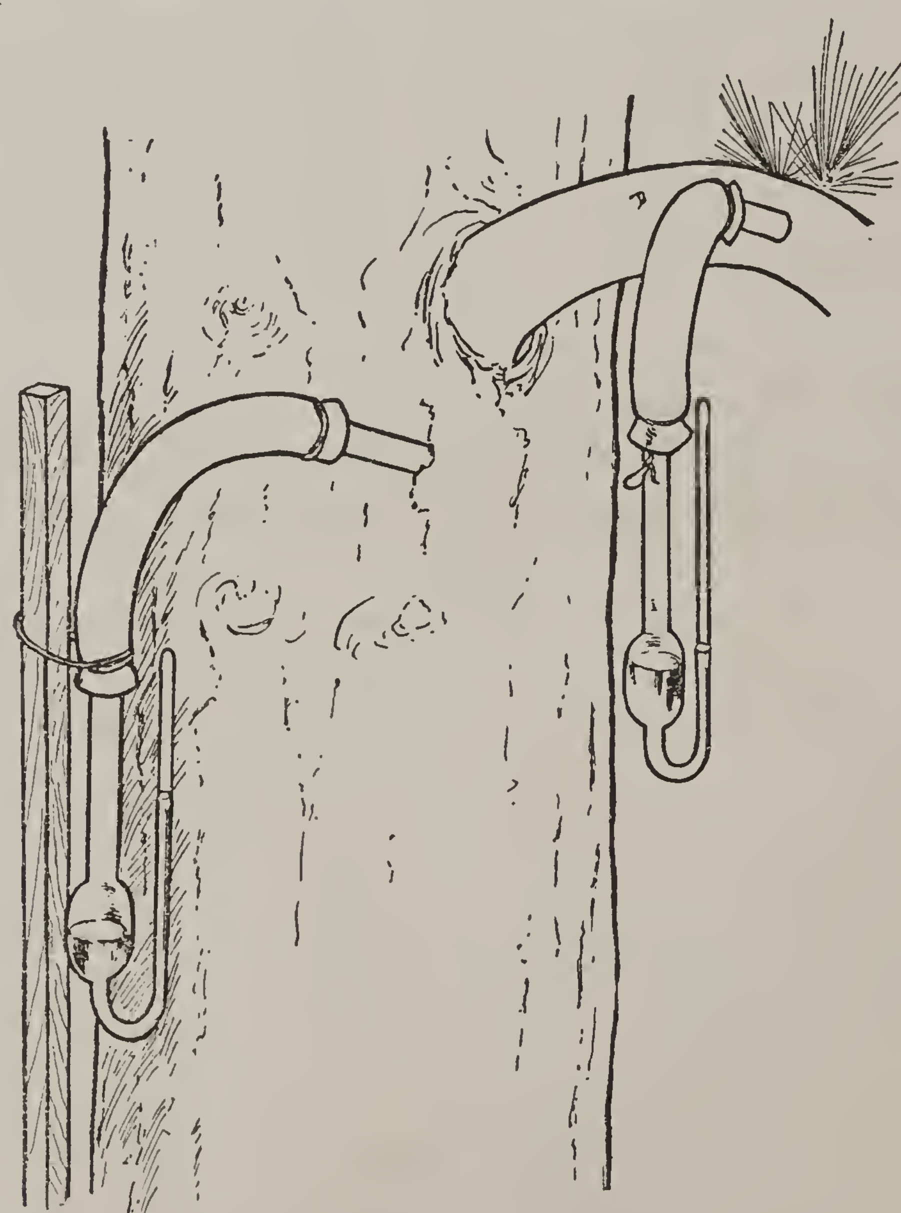

Fig. 9.—Manometer connected with a short section of metal tubing screwed tangentially into the trunk of a Monterey pine, showing positive pressure of about 2 atmospheres. A similar apparatus inserted in a large branch is also shown.

maximum pressure of 3.5 atmospheres had been registered by a manometer connected with a hole in the base of the trunk a few days previously, and 2.8 atmospheres, from a hole near the base of the branch to be tested. The branch was cut off within 8 cm. of its base, exposing a cut surface about 22 mm. across. A pressure-hose was clamped about the stump and connected with a manometer with closed end. Readings were made as set out in table 9.

TABLE 9.

Date.	Time.	Length of air column in manometer.	Pressure in mm. Hg.
		mm.	
Sept. 18	2 p. m.........	70	
	3 p. m.........	74	− 4
	3^h 10^m p. m....	77	− 7
	3 50 p. m....	80	−10
Sept. 19	8 a. m.........	78	− 8
	11 a. m.........	78	− 8
	4 p. m.........	82	−12
Sept. 20	8 a. m.........	78	− 8

Irregular but diminishing negative pressures were shown in the following week, but at no time was a positive pressure set up.

Monterey pine No. 17, which was still in a condition of enlargement of the trunk late in September, was chosen for the semi-final test of the exudation pressures from the cut end of a branch and from

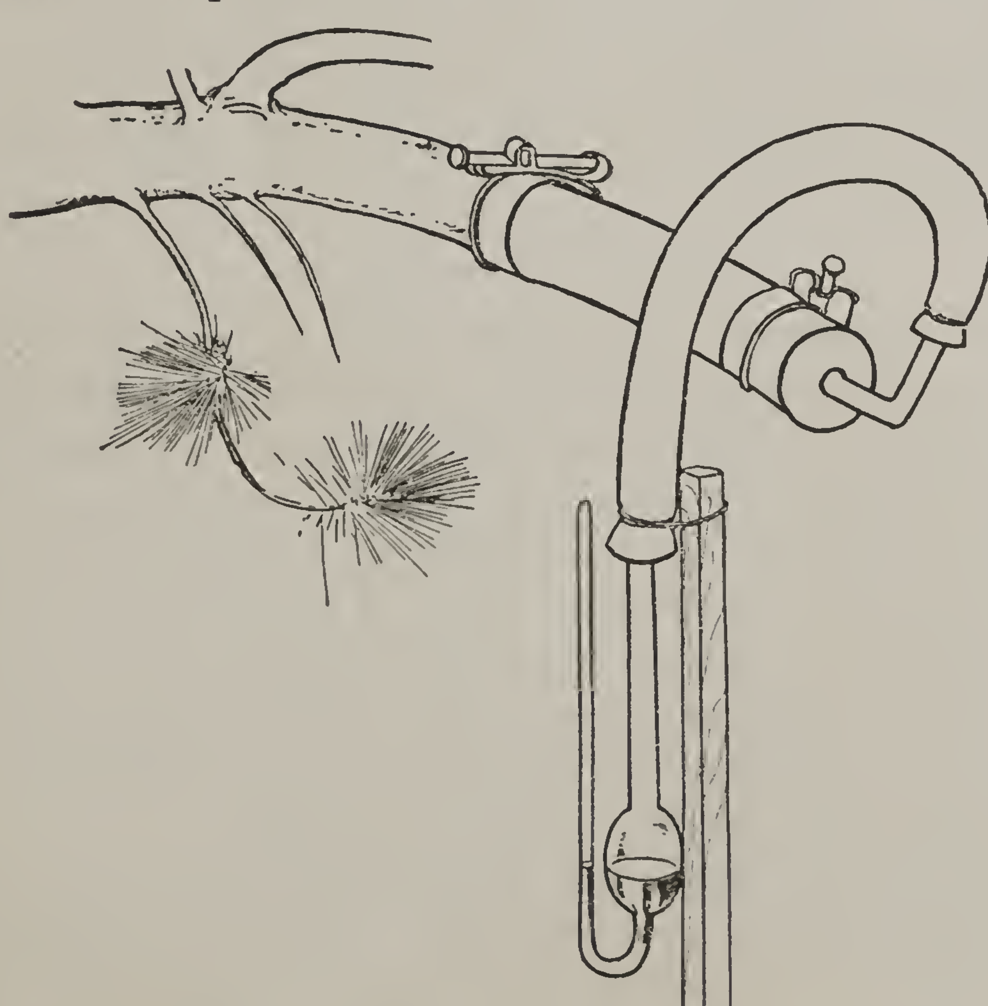

FIG. 10.—Manometer connected with cell clamped over the end of a large branch of Monterey pine No. 17.

a layer of wood. A brass tube was screwed into the trunk at a height of about 50 cm. from the base and connected with a manometer with a closed end. A branch arising about 10 cm. from the point of attachment of the above-mentioned instrument was cut off at a distance of 1.2 meters from its base, the stump being about 22 mm. across. A section of pressure-tubing was clamped securely over the stump and connected with a manometer with a closed end. (Fig. 10.) The two settings were strictly comparable in every way. Readings are given in table 10.

Table 10.

Date.	Time.	Length of column of air in manometer on trunk.	Length of column of air in manometer on branch.
		mm.	*mm.*
Sept. 25	11^h 15^m a. m.	105	
	11 20 a. m.	[1]	110
	11 30 a. m.	103	114 = −4
	12 noon	105	120
	2 p. m.	110 = −5 mm.	122 = −12
	4 p. m.	101 = +4 mm.	118 = −4
Sept. 26	8 a. m.	65 = 1.6 atm.	110 = 0
	10 a. m.	63 = 1.68 atm.	118 = −8
	12 noon	61 = 1.7 atm.	120 = −10
	2 p. m.	60 = 1.75 atm.	123 = −13
	4 p. m.	54 = 1.9 atm.	114 = −4
Sept. 27	9 a. m.	47 = 2.2 atm.	110 = 0
	10 a. m.	47 = 2.2 atm.	110 = 0
	12 noon	46 = 2.3 atm.	113 = −3
	2 p. m.	46 = 2.3 atm.	117 = −7
Sept. 28	12 noon	45 = 2.3 atm.	111 = −1
Sept. 29	8 a. m.	44 = 2.4 atm.	103 = +7
	12 noon	45 = 2.3 atm.	107 = +3
Sept. 30	8 a. m.	46 = 2.3 atm.	104 = +6

[1] Negative pressure for 5 min.

The above experiment was duplicated on another tree a month later. The manometer connected with a hole bored in the trunk showed a positive pressure of 2 atmospheres 40 hours later. The air column in a similar manometer attached to a branch arising near the point of attachment of the instrument noted above was lengthened from 105 mm. to 112 mm. within a half hour by negative pressure or absorption of water from the system. This was gradually reduced so that the mercury returned to zero 2 days later and a few readings were made in which the air column was compressed to 104 mm. which would be a very slight positive pressure.

The course of the pressures registered by the two instruments makes it obvious that the factors dealt with in the trunk and branch are not identical. The positive or negative pressure of the branch at any time is of course a resultant. The active cells near the exposed surface would pour their exudate into the water and tend to set up

a positive pressure. At the same time, the internal layers of wood and the broken-down pith offer some arrangements whereby water may be absorbed from the liquid bathing the surface. Absorption in this way was greater than exudation during the first three days of the test, but on the fourth and fifth days some positive pressures were set up.

Manometers were attached to the trunks of small trees of *Salix lasiolepis* and *Juglans major* at the same time the above measurements were being made. Negative pressures of 2 mm. Hg were shown during the first day by *Salix* and of 5 mm. Hg by *Juglans*. Positive pressures of 1.05 atmospheres were shown on the following days by *Salix* and of 1.1 atmospheres by *Juglans*. No attempt was made to compare these results with measurements by manometers attached to stumps.

EXUDATION PRESSURE IN ROOTS.

The soil was cleared away from a large lateral root 10 cm. in diameter, 1.3 meters from the base of a large pine near No. 2, and in about the same condition as No. 1, except that the soil around it was very dry. A manometer with a closed end was affixed to this root by a threaded brass tube screwed into a hole about 5 cm. deep. Water was put into the cavity the moment the auger-bit was withdrawn and the gage was in order within 5 minutes. Water was immediately absorbed by the root, as was shown by measurements in table 11.

TABLE 11.

Date.	Time.	Length of air column.	Date.	Time.	Length of air column.
		mm.			*mm.*
Sept. 3	$3^h\ 15^m$ p. m.	120	Sept. 5	$8^h\ 30^m$ a. m.	87
	3 20 p. m.	121		11 30 a. m.	85
	3 30 p. m.	122		5 15 p. m.	84
	3 45 p. m.	121	Sept. 6	9 a. m.	82
	4 10 p. m.	121		2 p. m.	81
	5 p. m.	120		5 p. m.	81
Sept. 4	$7^h\ 30^m$ a. m.	104	Sept. 7	11 a. m.	82
	9 a. m.	104		$4^h\ 30^m$ p. m.	82
	11 a. m.	105			
	2 a. m.	103			
	$3^h\ 15^m$ p. m.	102			

The soil around the base of this tree had reached a lower limit of less than 4 per cent of soil moisture some weeks before and the roots were in an inactive condition. "Negative" pressure was shown as a result of initial absorption of water from the tubes when first attached. The highest pressure developed was nearly 1.5 atmospheres.

The instrument was next attached to a large root of Monterey pine No. 1, which had been irrigated in midsummer and which was in a condition of active growth. The root showed daily reversible

variations by which it increased in diameter during the daytime and contracted at night, as described elsewhere in this paper. A hole was bored in the root between the point of dendrographic contact, as also described on page 35, and the base of the tree. This cavity had a capacity of about 5 c. c. Connection with a manometer was made in the usual manner. Readings were obtained as shown in table 12.

Table 12.

Date.	Time.	Air temp.	Root temp.	Length of column of air in manometer.
		° *C.*	° *C.*	*mm.*
Sept. 8	9 a. m.	15	13	76
	10 a. m.	17	15	75
	2 p. m.	18	16	75
	4 p. m.	17.5	17	75
Sept. 9	8 a. m.	14	14	47 = 1.3 atm.
Sept. 10	7 a. m.	14	14	41 = 1.85 atm.
	4 p. m.	18	17	46
Sept. 11	9 a. m.	17	17	47
Sept. 12	2 p. m.	18	17	51
Sept. 13	8 a. m.	14	14	55
	12 noon	17	17	54

The apparatus was taken down and attached to a new hole about 25 cm. distant and toward the tip of the root. The maximum pressure was reached within 48 hours after the beginning of the operation. The readings are given in table 13.

Table 13.

Date.	Time.	Air temp.	Root temp.	Length of air column.	Remarks.
		° *C.*	° *C.*	*mm.*	
Sept. 13	12 noon	17	17	76	No "negative" pressure.
	$3^h\ 30^m$ p. m.	18	18	72	
Sept. 14	1 30 p. m.	17	17	40	1.8 atm.
	5 30 p. m.	15	15	39	1.84 atm.
Sept. 15	8 a. m.	15	15	35	2.17 atm.
	$2^h\ 30^m$ p. m.	17	17	35	2.17 atm.
Sept. 16	8 a. m.	17	17	37	

The results shown in table 13 are of unusual interest, since the measurements made by all investigators from the time of Hofmeister have led to the conclusion that exudation or bleeding pressures were not marked in the Coniferæ. Pitra found pressures of 114 mm. of mercury in branches of *Pinus sylvestris*,[1] but so many observers failed to get

[1] Pitra, A. Versuche über die Druckkraft der Stammorgane bei den Erscheinungen des Blütens und Thränens der Pflanzen. Jahrb. f. Wiss. Bot., 11, 1877.

positive results that Jost summarizes knowledge concerning this matter, as follows:

Bleeding-pressure, however, in all Coniferæ is extremely feeble, as very many estimates clearly prove. Hofmeister (1862), indeed, states that as a general rule Coniferæ do not exhibit the phenomena of bleeding; and although Wieler (1893), at a later date, was able to show that bleeding could be demonstrated in these plants, still it is impossible to draw any other conclusion than that it was quite insignificant in amount.[1]

The seat of exudation pressures is in the parenchymatous cells in contact with the tracheids, and the possibility of the expression of the turgidity of these elements in forcing water into the wood cells has been at no time excluded. Failure to detect or determine pressures in such stems may be attributed to a faulty technique. Thus, for example, the excision of a shoot and the attachment of a manometer to the stump would destroy the cohesive column of water, and bring to a standstill the solutions moving past the cells, with the result that the solutions in these cells would soon receive such an amount of organic matter as to be isotonic with the living cells. Holes bored into the trunks of slowly growing pines which lay down only a thin layer of wood each year would extend into the older wood, and if the connecting or outlet tubes were fitted to the openings by means of rubber stoppers, the stopper would be thrust in through the outer active layers into the older wood, in which the column of water probably no longer existed. Some additional difficulty would also be encountered in the presence of resins.

The successful outcome of the observations as noted above may be held to depend upon two features, the choice of material and the method of attachment of the tubes to the bore made in the tree. The Monterey pine forms each year layers of wood 2 to 10 mm. in thickness; the greatest conduction takes place in the wood of the previous year, and the cohesive column of water and living cells are present in the third or fourth layers also. Exudation pressures may therefore be expected to develop in an external shell of the trunk as much as 2 cm. in thickness. The metal tube with which attachment was made to the tree was screwed into the bore only so far as to secure firm attachment, penetrating to the wood of the previous year. Canada balsam was used to seal around this tube to prevent drying of the edges of the bore, and the resin which pours into the bore quickly seals the tube in place and prevents leakage even under the high pressure noted above.

The pressures noted above are exceeded only by that of about 9 atmospheres found by Boehm in *Aesculus*, Figdor 6–8 atmospheres in tropical trees, and Molisch in native Austrian trees.[2] Other maxi-

[1] Jost, L. Lectures in plant physiology. Translated by R. J. H. Gibson, 1907. See p. 62.

[2] Boehm, J. Ueber einen eigenthümlichen Stammdruck. Ber. d. Deut. Botan. Ges. 10. 539-544. 1892.

Molisch, H. Ueber localen Blutungsdruck und seine Ursachen. Bot. Ztg. 60. 45-63. 1902.

mal records are those of Wieler, who found pressures of 139 cm. of mercury, less than 2 atmospheres, in *Betula alba*, while Clark had previously found pressures of 192 cm. of mercury, 2.5 atmospheres, in *Betula lenta*.[1]

HYDROSTATIC SYSTEM OF MONTEREY PINE.

The water system of the tree terminates in the living cells of the leaf, which, losing water to the air, renew their supply continually by endosmotic withdrawal from the xylem elements which run to them. By the widely accepted scheme accredited to Professor Dixon the water in the xylem elements, wood-cells, and other elongated elements forms a continuous column down through a cylindrical shell of such conducting elements to the lower end of the tree, terminating in the xylem elements in the roots. This column, consisting of strands of water continuous through a large number of elements, is under the tension set up by the pull of the osmotically active living cells at the upper end of the system, and can exist only in wood cells which contain no air or gas bubbles. The final or ultimate force which keeps this column in a state of tension is the evaporation from the exposed surfaces of the living cells. Such a pull, of course, results in the upward movement of the water in the column, or in the "ascent of sap." With the mechanical conditions fulfilled by the presence of the continuous column of water, and with the terminals of the xylem in contact with the transpiring cells, the tension of the water column may be maintained and sap may move upward in dead trees, as has been successfully demonstrated more than once. After a tree dies, however, the transpiring cells begin to deteriorate, so that a stage is soon reached in which the pull set up by evaporation from their surfaces is no longer conducted direct to the xylem and the movement stops.

Emphasis is to be laid on the fact that it is not proven that the action of *living* cells at the top of the tree is necessary for the ascent of sap, but that the xylem with its continuous column of water terminates in a mass of gelatinous elements which may lose water from its free surfaces and replenish the water-supply from the xylem cells in contact with them. If the water column were taken to exist as a "Jaminian chain," or as passing through wood cells in which a gas bubble existed, the presence of perforations in the membranes of the pits in the wood cells would be a matter of great importance, since, as Professor I. W. Bailey has demonstrated, a tension of two or three atmospheres would pull air through these perforations and thus break the column. It seems fairly evident, however, that the ascent of sap is chiefly or almost wholly in wood cells in which no air exists. No elongated elements beside the resin ducts are in the Monterey pine. The problems of the ascent of sap in the wood of the Monterey pine is

[1] Pfeffer W. The physiology of plants. Trans. by A. J. Ewart, 2d ed., 1, 259. 1900.

therefore wholly one of the mechanics of movement through a system made of wood cells, as described on page 9, which have an average length of about 4 mm.

The matter of the possible participation of living cells in the upward movement of sap is to be considered in connection with the presence and action of the parenchymatous cells of the xylem, including the medullary rays. The mass of information now available gives but little support to the proposal that the forces which cause solutions to rise in the wood cells to the tops of trees originate chiefly in these living cells. A few writers, however, who claim space in scientific publications, still spread hopelessly inadequate explanations of the pumping action of living cells as the force which sends solutions to the tops of trees at a high imaginary rate. No refutation or discussion of such claims seems necessary.

Active and turgid living cells are, however, in contact with the xylem and form part of the conditions under which the water column extends from the roots to the leaves. The possible effect of the action of these cells on the solutions is a matter of some interest. The parenchymatous cells of the xylem have been found to show an osmotic equivalence of about 3.5 atmospheres. The arrangement of the xylem parenchyma cells is such that many of them are in contact with or have a common wall with wood cells so that a pit in the wall of the wood cell will be bordered internally in the tracheid but not inside of the parenchymatous cell. The membrane of this incomplete pit may be taken to be much more readily permeable to solutions than the remainder of the wall, so that water and some materials in solution from the turgescent cell may pass through it into the wood cell.

It might be argued that if the parenchymatous cells are capable of showing such high pressures when connected with a manometer, liquid would be forced into the wood cells to an amount which would make the action of these living cells the principal agency in forcing sap upwards. It has been found, however, that the conducting tracts are in a state of tension by which they contract when the upper end of the shoot is removed and the pull on the water column in the wood cells is removed. If the thin-walled cells are in a state of action by which water is continually forced into the wood, it is at such a rate as to be inadequate to meet the pull from the leaves.

When cavities are bored in the trunk similar to those to which manometers are fitted, and filled with dye solutions, the color diffuses downward away from the cavity at a rate which was found to be about one-sixth of that at which it passed up the stem. The upward movement, which is at the rate of 2 to 6 cm. per hour, ordinarily is probably very much more rapid for the first half-hour. This movement is one connected with the water column, and the water used in the manometer must be visualized as being drawn into this stream. This results

in some cases in a "negative" pressure, which after an hour or two is taken up and a positive pressure due to excretions poured into the cavity is set up.

The reduction or slowing down of the transpiration stream, such as must occur when a deciduous tree casts its leaves, when the leaves of a conifer take on the autumnal and winter condition, in lesser degree when the stomata under their diurnal closing or narrowing, or when a pine is defoliated, would result in a lessened tension on the column of water in the wood cells which might become nearly stationary. In this case the exosmotic action of the living cells would tend to equalize the liquid in the xylem and in the living cell. An analysis of the cell-sap a short time after artificial defoliation of a pine would doubtless show a higher organic content of the liquid in the wood cells.

Dixon and Atkins found that the osmotic potential in various trees, due principally to sugars and non-electrolytes, increases in concentration upward in the stem in the spring. A minimum is found at the 4-meter level in the autumn in the maple, the concentration increasing toward the terminal of the shoot and of the root. Electrolytes show a similar arrangement in the autumn. Naturally, the least difference in osmotic potential was found in the resting-season. Sugars were at the lowest concentration in late spring, but electrolytes were highest at this time.[1]

The ascending electrolytes which have gained access to the sap in the roots would pass into the parenchymatous cells and cambium, according to the mobility of the ions and the nature of the walls and plasmatic material encountered, as has been demonstrated in a variety of experiments, although the manner in which the accumulation of certain elements such as potassium and calcium accumulate in solution in living cells is yet to be made out.[2]

On account of the reflex action of pressure itself on osmosis, it may be expected that when exudation is calibrated by the use of a U-tube or open-arm manometer the resistance or pressure encountered by the exudate will begin at zero and increase with the amount of the exudate accumulated. On the other hand, when a closed-arm manometer is sealed into a hole bored into a trunk, the resistance quickly reaches the maximum, and with the exudation of only a few cubic centimeters of liquid. The course of the flow and pressure measured by the two methods show a difference caused by the effect of the head of pressure on the osmotic action of the parenchymatous cells. The main features of the action are determined by the volume of the cells which are given outlet by the operation or experiment and their osmotic equivalence. In the case of the Monterey pine, resin canals

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[1] Dixon and Atkins. Osmotic pressures in plants. Sci. Proc. Roy. Dublin Society, 15, March, p. 51. 1916.

[2] MacDougal, D. T. Permeability and the increase in volume of contents of living and of artificial cells, 62, 1–25. 1923.

which are in a state of tension by the action of the thin-walled cells about them also pour their contents into the cavities or cuts made in the stem and contribute to the volume of the flow which may be measured. How far imbibition or osmotic action of the resinous material immersed in the water introduced into the bore may contribute to the pressure is not known. The presence of this material soon clogs the apparatus and seals the pressure system within three or four days, as described in the previous pages.

A graph which would express the rise and decline of the pressure or the outflow includes no features which can be safely interpreted as daily variations. Changes in temperature may affect osmotic action in the cells, but the rise which would increase osmotic pressure also causes expansions in the column of mercury and of compressed air in the mercury of the manometers, constituting errors which can not be accurately calculated. Daily variation might be more easily detected in plants in which elongated vessels bring into operation distant cell-masses when a stem is cut or bored.

It was also found that no analysis of the measurements could be made which showed any connection between the rise and decline of the pressures with the daily reversible variations in stems. Some coincidences in low readings are apparent, but no causal connection can be established between the contracted or expanded state of the woody tissue with the rise and fall of the pressure. The pressure in a closed manometer will show an apparent drop after midday, due to high air-temperatures and the consequent expansion of the inclosed and compressed column of air, but this lessened pressure is not apparent until contraction of the wood cells has been in progress may hours.

The young roots present an arrangement by which the endodermis, cortical cells external to it, and active parenchyma cells internal to it, constitute a complex membrane through which water is drawn osmotically into the interior of the root and forced into the xylem in same general manner as into the wood of the stem. The endodermis forms an unbroken layer, the cortical tissue shows intercellular spaces, and the xylem parenchyma abuts on the non-living cells so that water drawn through this complex membrane is forced into the conduits leading upward to the stem. The operation of such a system is dependent upon the presence of elements with a higher osmotic value internal to the endodermis than external to it and with an equivalence greater than that of the soil solution.

The electrolytes of the soil solution pass through this layer and into the sap of the xylem by their own diffusive action. In so doing each one modifies the colloidal condition of the cells in such manner as to affect the relations of the complex membrane of which they form a part in such manner as to affect the passage of the others.[1] This is a

[1] MacDougal, D. T. The arrangement and action of material in the plasmatic layers and cell-walls of plants. Proc. Amer. Phil. Soc., 63, 76. 1924.

matter which has been dealt with by various workers concerned with the effects of various salts on absorption and with interferences or so-called antagonisms. The material which gives the cells internal to the endodermis their comparatively high turgidity may be sugars or other compounds which have diffused downward through the stem to the root, organic acids derived from these sugars, or, as Priestly has suggested,[1]

> The disintegration of the plasmatic contents of the maturing xylem may furnish some material which would be of contributory value in establishing the osmotic gradient from the soil solution to the interior of the root.

How far the pressure of the osmotic action of the endodermis forces water toward the base of the stem of a Monterey pine or upward is not known. The fact that the conduits of even small plants which may not be more than a meter in height are in a state of tension by which they expand when transpiration stops suggest that the pressure set up in the endodermal membrane does not exert any serious pumping action. The exudation pressure measured in the thickened root of the Monterey pine was somewhat slower in reaching the maximum head, but otherwise presented no features essentially different from those of the stem. Such pressures are not to be confused with those originating in the endodermis. When manometers are affixed to large roots as described on page 55 the pressures which may be registered may be taken to be due to the action of the xylem-parenchyma, resin ducts and wood cells as in the trunk. Consequently it can not be assumed that water is being forced upward into the trunk at the "root-pressure" registered. In conjunction with this is to be noted the daily expansion of the root by which it becomes larger at the time of maximum water use by the tree. This increased size possibly rests on the greater proportion of parenchymatous cells in the secondary tissues of a large root. The daily rise in temperature would result in such cells assuming a higher degree of turgidity. If the wood cells are in a state of contraction, the shrinkage is masked by the action of the parenchymatous cells. It is conceivable that a head of osmotic pressure may exist at varying levels in the roots or even in the stem. This suggestion received additional support from the fact that the preliminary calibrations of a root of *Sequoia sempervirens* only a few centimeters in diameter shows that it contracts during the daytime, indicating that the head of pressure set up in the endodermal membrane does not reach this level in the system. (See p. 41.)

EFFECTS OF GIRDLING.

The voluminous literature of girdling includes but little information bearing directly upon the effect of the removal of the bark, cambium, etc., on the conductive action of the tracheids internal to such a

[1] Priestly, J. H. The first sugar of photosynthesis and the role of cane sugar in the plant. The New Phytologist, 23,255–265. 1924.

stripped zone. Strasburger describes the presence of droplets of resinous material in the tracheids of the conifers, and that the membranes are sometimes appressed to one side of the pit by this material.[1] Minute masses of resin have been seen in the tracheids and closing the pits of the Monterey pine, as described on page 66 of this paper. How much the formation of resin in tracheids may be hastened by girdling has not been determined.

Whatever this effect may be in the Monterey pine, it is not always marked and immediate. I have already described the girdling of a pine tree 22 years old, near its base, and noted the fact that the removal of the bark and tissues to expose the wood cells of the previous year did not materially alter the nature or course of the daily reversible variations. The girdling was carried out in May.[2] Not until the following year did the character of the daily reversible variations show any marked departures from the usual program. No regeneration occurring, this tree dies at the end of the season following the year of the operation. It would seem in this case that the cohesive column of water was not disturbed and that the death of the tree about 16 months after girdling might be attributed to disturbances in the downward movement of nutritive material.

The removal of the bark and newly formed cell-masses external to the wood in the upper part of a young tree gave results of a different kind. A zone 15 cm. wide about 2.3 meters above the base, and *above* a dendrograph which had been attached to the tree for some time, was exposed in March 1923. Growth was checked and reversible enlargements came down to a minimum. Ten days later reversible variations were again discernible and growth was resumed on the fifteenth day. The resumption of the former behavior of the trunk was accompanied by the formation of a callus over the girdled zone. The cores taken later showed that the callus had formed a layer about 2 mm. in thickness. It is highly probable that the girdling had disturbed the water column in the bared layer of wood, which was not more than 2 mm. in thickness. The temporary nature of the disturbance suggests that it may have been chiefly to such effects as those described by Bode,[3] in which it was seen that the touch of a metal point on the outside of a trachea caused a rupture in the water column inclosed. How this may be restored is not known.

The breaking of the continuous and complex column of water in the trunk of a tree is doubtless always followed by marked disturbances. If the column be restored, as has been done by various experi-

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[1] Strasburger, E. Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. See section on "Der Abschluss offener oder todter Stellen an der Wasserbahn, pp. 770–773. 1891.

[2] MacDougal, D. T., and F. Shreve. Growth in trees and massive organs of plants. Carnegie Inst. Wash. Pub. No. 350. 1924. See pp. 13–15.

[3] Bode, H. R. Beiträge zur Dynamik der Wasserbewegung in den Gefässpflanzen. Jahrb. f. Wiss. Bot., 62, 92. 1923.

menters, the stem or branch may resume activity after the normal manner. A complete disruption of the water column in the Monterey pine has been produced by bathing a zone of the trunk with boiling oil, which dissolves out but little material and waterproofs the treated zone against direct desiccation. A small encircling reservoir of sheet lead clamped to the trunk of a tree was filled to a depth of 20 cm. with boiling oil and maintained at a temperature of 100° C. for 2½ hours. Growth was recorded by the dendrograph a half meter above the treated zone for a subsequent period of 20 days, and the daily reversible variations continued for 5 days more, then flattened out. A control tree was treated in the same manner. An examination 48 hours later showed that the corky layers of the bark remained intact, the cambium was destroyed, and also the cortical and ray cells. The trunk below the treated zone had lost none of its water, two samples showing an average of 60 per cent of moisture. The sections taken from the outer layers of the treated zone showed an average of less than 30 per cent, one sample being as low as 25 per cent. The wood of this region felt dry to the touch and the water column was undoubtedly broken.

Five weeks after the treatment, when it became apparent that the trunk was in a state of permanent contraction, the tree under the dendrograph was supplied with some water by dipping the ends of cut branches in flasks. About one-fourth of the contraction was regained during the next 2 weeks after absorption by this method could not be restored. 5 liters of water had been taken in by the branches in 2 months.

Progressive desiccation and death of the tree from the treated zone upward now became apparent, so that at the end of September, 3 months after the treatment, green leaves were seen on the upper branches only, and these were on terminals, having been formed during the earlier part of the same year. When the tree was taken down several months after the treatment, the zone which had been heated was distinctly drier to the touch than regions above and below. The proportion of moisture appeared to increase upward toward tbe leaves, now in a dying condition, as denoted by their yellowish tinge.

A further test was made with a small pine tree 10 cm. in diameter in October 1923. A pair of wooden blocks was clamped around the tree for a foundation. On this was shaded a shallow vessel of plaster of paris about 5 cm. deep and holding about 50 c. c. of liquid. After the plaster was hardened properly, olive oil was heated in a dish and poured into the vessel encircling the trunk. The oil slowly escaped through a glass tube set in the plaster, and as it flowed out hot oil was poured in in such manner that the trunk was subjected to a temperature of 90° to 110° C. for half an hour. In addition, the plaster became warmed and a zone below that reached by the hot oil also probably was subjected to a killing temperature. It was estimated

that a total zone 10 cm. long may have been killed. The blocks and plaster were now removed.

The stem at the place of treatment, the limits of which were denoted by notches in a wooden block, was 6.5 cm. in diameter, and it was estimated that the age of the tree was 14 years.

On January 18, 1924, the tree had not changed materially in appearance, except that the treated zone was shrunken. With the oncoming season of growth and activity of the leaves the disturbance to the conducting system became apparent. The tree was completely dead by May and the leaves, which were fully dead, began to fall in June. The stem was cut near the base, and this part of the trunk was heavy and had a high moisture content; above, it was very dry, and when set in solutions of dyes almost no penetration ensued.

The above experiments were made during the summer months, when growth had slowed down or ceased, and transpiration was doubtless going on at a rate customary in the autumnal stage of the conifers. An assumed complete disruption of the water column accompanied by changes due to heat in the wood cells resulted in the death of the tree by a stoppage of the upward movement. Whether an actual stoppage of the tracheids took place, or whether substances were brought into solution in the sap as a result of the heating, which might exert a poisonous action on living cells, is not known. It would seem probable that of these two contingencies the changes of resinous material in the cells might well block the pits in such manner that a disturbed column of water could not be restored to normal, as was done experimentally by Professor Dixon.[1]

The experiment in which girdling on the upper part of the trunk was followed by a temporary disappearance of the daily reversible variations below are explainable only on the basis of the supposition that the operation interrupted the water column to an extent which was recoverable, or that direct transpiration from the surface made such a serious drain on the water column as to reduce it to a minimum which would not be affected by the action of the leaves.

The fact that Curtis found that girdling reduced transpiration in other woody plants leads to the supposition that a similar diminution took place in the pine, with a resultant disappearance of the daily variation.[2] No physical basis for such a lessening water-loss could be found in the present instance.

In the endeavor to ascertain what alterations might follow girdling, which would be of importance in this connection, a number of small trees were girdled a few days before September 1, 1924. On September 3, one from which a zone of cortex and bark 8 cm. in width had been

[1] Dixon, H. H. Transpiration and ascent of sap in plants. By MacMillan Co., London, 1914. See especially pp. 54–68.

[2] Curtis, O. F. The effect of ringing a stem on the upward transfer of nitrogen and ash constituents. Amer. Jour. Bot., 10, 361. 1923.

removed was cut down, the top taken off, and coupled to a Nelson pump. The base had been kept moist, and it was set in a vessel containing fuchsin solution. The pump was operated for 4 hours at a pressure of 740 mm. Hg. At the end of this time the stem had taken up 250 c. c. of the dye and 165 c. c. of clear sap had been collected in the first flask in the series leading from the plant to the pump. This sap had a pH of 6.1 and contained 0.26 gram reducing sugar per 1,000 c. c. of liquid. The dye had been pulled up past the girdle in all of the layers of wood, and no special variation of the conduction stream was noted. It was found, however, that in stems, after a longer time had elapsed after girdling, an accelerated development of resinous matter in the wood internal to the girdle had taken place.

It is known that similar formation of gums and tyloses may fill the wood cells in various plants in the region of unprotected girdled areas on woody stems. In the case of the pine it is to be noted that the exposed surface in some cases is coated within a few hours by resinous material.

Some of the young girdled trees noted above were allowed to stand for about 2 weeks and were then taken for experimentation. One with a diameter of 3.5 cm. at the base, 2 meters in height, and with a heavy leafage was cut on September 18. The girdle was 5 cm. in width, with its lower margin 40 cm. from the base of the tree. The base, kept moist, was stepped into a vessel containing 250 c. c. of fuchsin solution at 3 p. m. on the 18th. At 9 a. m. on the 19th, 18 hours later, 200 c. c. of the solution had been absorbed. The dye had gone up a distance of 140 cm., or at the average rate of about 8 cm. per hour. All layers were colored for more than half of this distance, and above this the dye was in the second and third layers only. When the stem was bisected it was seen that the exposed wood formed during the current year had not conducted the dye, which had come up in this layer to the margin of the exposed zone, been blocked out of it across the girdle, and had then diffused into and followed it up many centimeters above, as has been noted. The wood cells contained an abundance of resin, the bordered pits being blocked in many of them. The resinous excretion on the exposed surface of the girdle had dried in irregular masses, so that a heavy water-loss was possible.

The above conditions suggest that the accumulation of carbohydrates in the zone above a girdle, as I have described in the case of Monterey pine No. 14, may be simply a stoppage of a downward flow to which an active outer layer of wood may be necessary. An increased formation of wood in this region was noted.[1] O. F. Curtis has also seen something similar in the formation of heavier bark in the region above a girdle in *Ligustrum*.

[1] MacDougal, D. T., and F. Shreve. Growth in trees and massive organs of plants. Carnegie Inst. Wash. Pub. 350, pp. 14 and 15. 1924.

EFFECT OF REMOVAL OF TRANSPIRATORY MECHANISM FROM UPPER END OF WATER COLUMN.

The removal of the upper part of the main stem of a Monterey pine carrying away all or most of the branches and leaves lessens the transpiratory losses and removes the source of tension set up in the complex column in the trunk. As might be expected, the relief or discharge of this tension is followed by alterations in the daily reversible variations of the section of the trunk below. As the photosynthetic processes are also canceled, the downward movements of carbohydrates and of possible excitatory substances are also brought to an end. Exudation pressures are set up in the tissues near the cut surfaces and the division and growth of the cambium on the lower part of the trunk is altered, as has been described by Neef.[1]

I have shown in a previous publication that in consequence of the removal of all of the branches and leaves of a Monterey pine the ensuing disturbances noted above are followed by the death of the tree. The Monterey pine does not have the power of regeneration by which adventitious buds may start after such mutilation and thus replace the green parts. It is highly probable that young, vigorous trees might be found in which the buds were so near activity that shoots might be formed, but usually the tree appears to be in almost daily and continuous need of the transpiratory action and photosynthetic products of cells containing chlorophyll. The green bark of young trees does not appear to be capable of meeting this need. If a few branches are left, growth may continue and daily variations may be displayed which will be commensurate with the volume of the transpiration stream.

In the case of a young tree with relatively heavy branches near the base, one (No. 18) was decapitated 2.2 meters from the base, four large branches remaining above the dendrograph and three large ones below. This operation, performed in mid-season for growth, 1923, resulted in the immediate stoppage of enlargement of the trunk and the reduction of the daily variation, which had never been very large, as this tree stood in a very moist soil. (See fig. 11.)

No further growth was recorded under the dendrograph during the remainder of the season, but late in the summer some reversible variation was shown, the contraction in the morning being very abrupt, and the time during which the decrease in diameter took place being much less than in a normal tree. Some growth took place March to June 1924, at which time the daily variation became more pronounced. The amplitude lessened with the progress of the season after the course of behavior seen in normal trees. Another tree topped higher up, removing only about one-half of its leaf surface and allowing many vigorous branches to remain, showed but little change in the daily

[1] Neef, F. Ueber Zellumlagerung. Zeitschrift f. Botanik, 6, 464–547. 1914.

variations or in growth. All such experiments indicate a direct connection between the amount of possible transpiration and the reversible daily variations. These depend directly upon the changes in the tension of the complex column of water in the wood-cells.

This matter is illustrated still further by the behavior of Monterey pines Nos. 10 and 15. No. 15, which was 20 years old, was cut a height of 2.2 meters from the base in May, near the end of the growing season of 1923. No branches remained. Enlargement continued for 19 days, after which some shrinkage ensued, and the amplitude of the daily reversible variations lessened. After 2 months a terminal section of 6 cm. was found to be entirely dead and the proportion of water in the trunk 4 months after the operation was so low as to denote death of the entire stump. The lack of any form of continuing transpiration from a continuous column in the trunk was undoubtedly

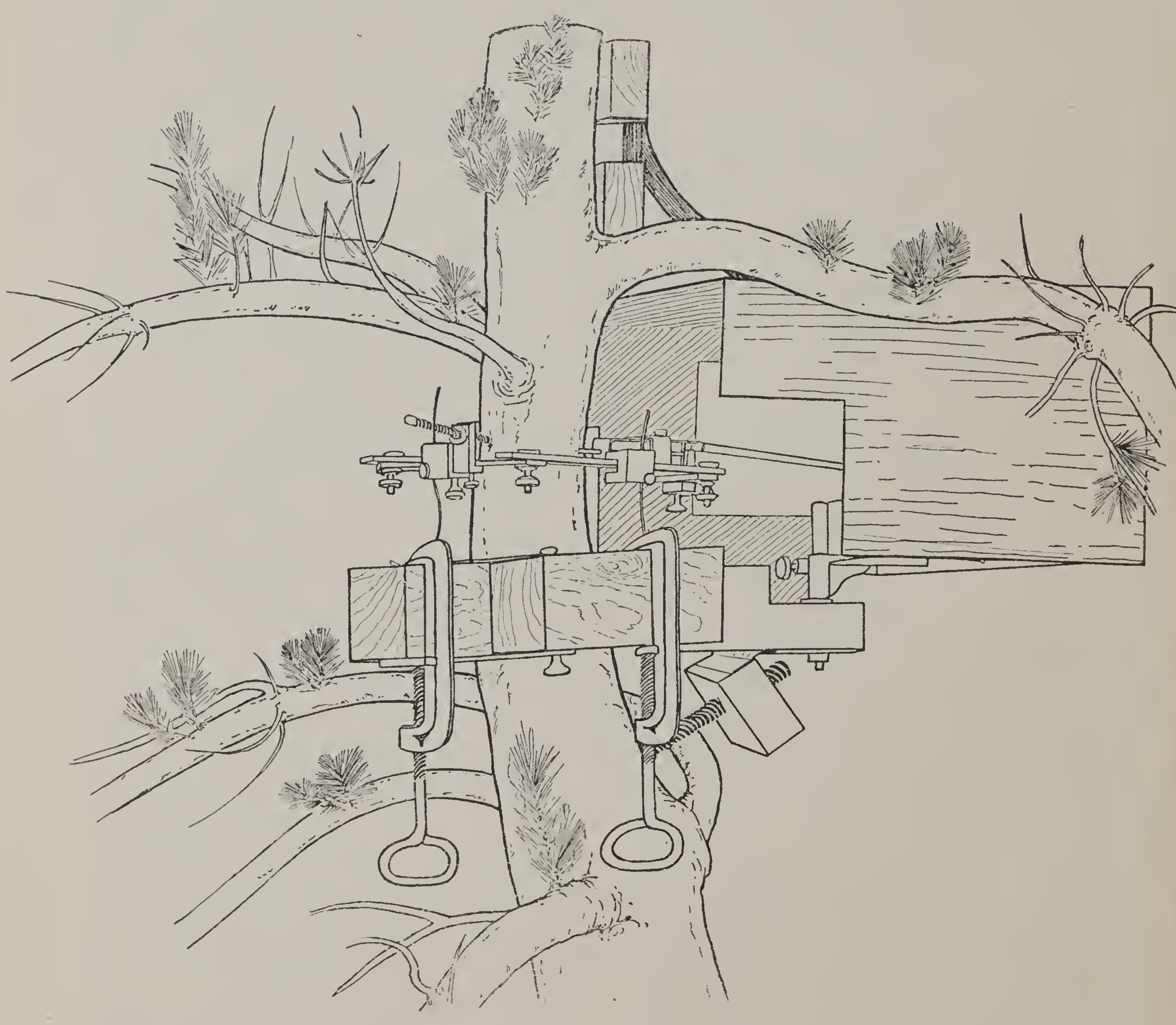

Fig. 11.—Monterey pine No. 18 after the top had been removed. Growth in the region in which the dendrograph was attached was at a minimum during the two following seasons.

a contributory factor, if not the main cause of the extinction of the tree.

No. 10, which was 25 years old and stood near No. 15, was cut 3 meters from the base, with several small branches attached to the stump. These were sufficient to maintain some tension in about three-fourths of the circumference of the tree. Growth following the cutting in May 1922 was not markedly different from that of the preceding year, although some disturbances of the daily variation attended the cutting and continued for two months.

Four of the branches died late in the season. The dendrograph had been arranged so that one bearing was on the sector subtended by the three living branches, and it was seen that growth was slow and uncertain in 1923, the total amount being very slight. Daily reversible variations were entirely lacking in the latter part of the season. The trunk went through the months from September to January with no perceptible alteration in diameter and with almost no daily variation. On January 24, 1924, a fairly steady increase began which continued until February 18, at which time an addition of 0.5 mm. had been made to the diameter. In the earlier part of this period the daily fluctuations were of the usual type, but gradually flattened into long, gentle undulations with very small amplitude. In mid-April it became apparent that a slow shrinkage was in progress. An examination made during the first week in June showed that the lowermost of the three living branches was showing indications of deterioration, some of the branchlets bearing dead leaves. Next it was found that the bark was flaking away from the trunk on the opposite side from these branches, which were in a sector of about one-third or one-fourth of the circumference. A girdle cut in the dead bark above the dendrograph showed that the living strip of phloem comprised about 20 cm. of the circumference, which was 38 cm., 0.5 meter above the moist or living strip, narrowed to 9 cm., which was the width subtended by the branches, 0.5 meter below the dendrograph and within an equal distance of the base. The width of the sector of living tissue was much greater toward the base and at 30 cm. included the entire circumference.

The dendrograph was reset with the contact screw bearing directly on the dead wood on one side, while the sliding silica rod of the lever was in contact with the phloem. Fortunately, this bearing was in the middle of the living zone, directly below the branches. Cores taken with the increment borer showed layers which confirmed the estimates for the growth of the layers in 1922 and 1923. It was also apparent that the tree was alive around the entire circumference under the dendrograph during the growing-season of 1923, at least under the two opposed bearing points. The layer formed under the dendrograph in 1924 was about 1 mm. in thickness, from which it was evident that during the growing-season of 1924 the increase of one side under the

bearing arm of the lever had been partly compensated by the shrinkage and decay of the phloem on the opposite side. The shoots or new "candles" formed in 1924 were 2 to 4 cm. long, with the leaves appressed and making no more than half this length.

In July 1924, more than two years after the upper part of the trunk had been cut away and the dendrograph record had finally come down to a direct line with no measurable daily variation, a test was made to determine the presence of continuous water columns along which material might be moving upward through the trunk below the dendrograph. These holes were filled with a solution of fuchsin which was renewed through tubes attached to the openings. Five days later the trunk was cut down near the base, terminating the entire experiment. The dye had moved upward and downward to the distance of a few centimeters only and in about the same irregular manner in all of the layers, showing that no distinct channels or streams were in action and that the columns had been completely disrupted some time earlier in the season.

The extreme sensitiveness of the Monterey pine to disturbances which result from the stoppage of movement in the water column is to be remarked. In addition to the cancelation of the diffusion of material from the leaves toward the base of the stem, the upward movement of electrolytes is also brought to an end. The ascending sap, in addition to the organic material which finds its way into the wood and is consequently carried upward, also carries the electrolytes from the soil which have penetrated the external layers of the root and come into the xylem by their own diffusive action. Once in the non-living elements, these substances are carried upward at a rate of several centimeters per hour. During this upward passage the electrolytes diffuse in all directions, passing through colloidal walls in accordance with their own ionic mobility and modified by the nature of the wall and by mutual interferences. A continued supply passing upward and diffusing laterally at an absolute small rate may well be quite as important to the living cells of the phloem as hypothetical messengers or hormones from the leaves or other accessory substances. Emphasis is to be laid on the fact that breaking the moving water column by decapitation of a tree stops both photosynthesis and the resultant diffusion of its products, as well as the upward movement of the soil salts necessary for growing or active cells in the phloem.

EFFECT OF LESSENED TRANSPIRATION ON GROWTH AND REVERSIBLE VARIATION IN MONTEREY PINE.

The removal of the leaves of a pine tree takes away the photosynthetic apparatus, deprives the plant of food material which may be in the leaves, and reduces transpiration to a minimum. This was especially the case with the trees treated as described in the following

experiments, in which the leaves were pulled directly out of their sheaths, the scar being quickly sealed by the resinous exudate.

Only mature leaves were removed in some of the tests described below. Defoliation in January 1924 thus left the short extensions of the twigs with the young leaves; defoliation in March would find these leaves about half grown and the operation in June would leave the tree in the condition of having mature leaves formed during the current year. Defoliation in October was carried out so that all of the leaves were removed.

All of the leaves, including those formed during the current season, were also removed from No. 23 in 1924. Complete defoliation in

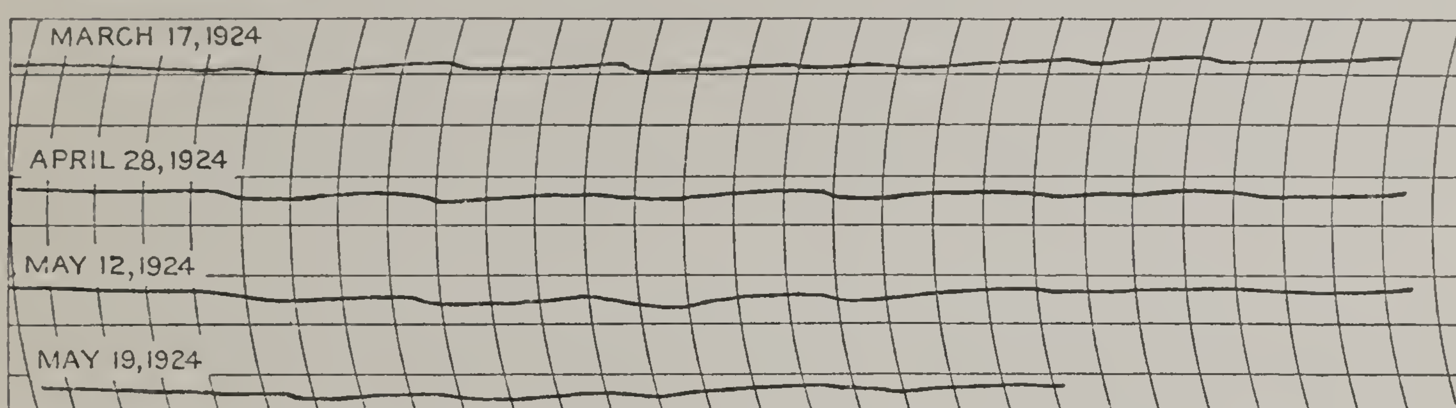

FIG. 12.—Dendrographic record of Monterey pine No. 25, during several weeks toward the close of the experiment, when the tree was supposedly dead, having been deprived of all leaves in the previous October. ×10. Intervals = 1 cm.

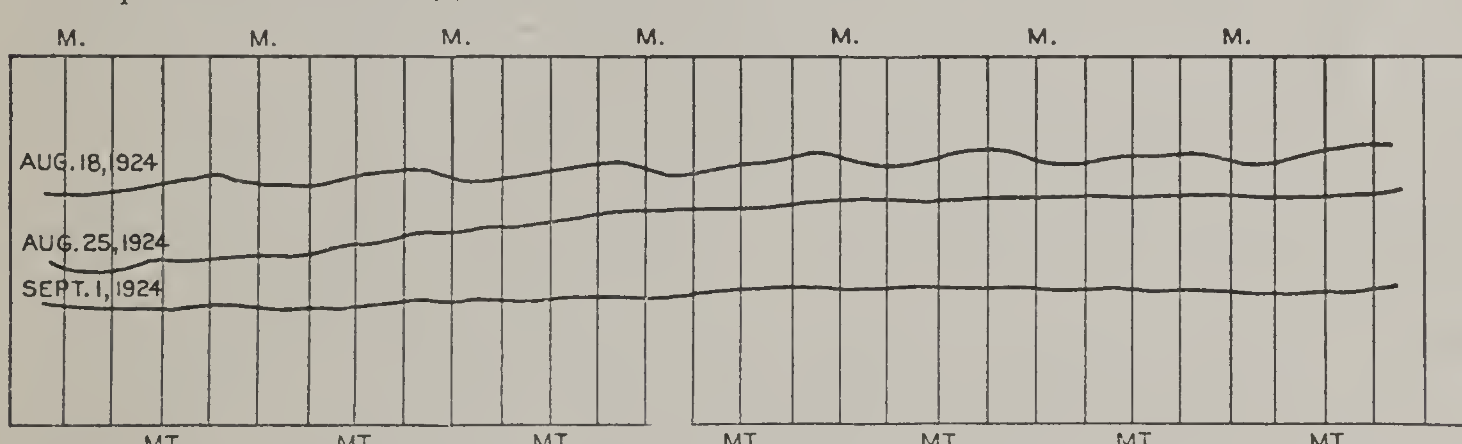

FIG. 13.—Dendrographic record of Monterey pine No. 23, which was completely defoliated on August 25, 1924. This was followed by continuous expansion for 10 days, with a subsequent reduction of the amplitude of the daily reversible variations. ×10. Intervals = 1 cm.

both cases resulted in the death of the tree within a short time. The details attending the performance of these operations are as follows:

A dendrographic record of Monterey pine No. 25 having been kept for a few weeks, the leaves were removed on October 1, 1923. The tree was 4 meters in height and the base of the trunk had a diameter of 5 cm., it being about 12 years old. The total weight of the leaves amounted to 4.2 kg. The rate of daily reversible variation at the time of the operation was very high, amounting to 1 part in 170 of the diameter. The day following defoliation the variation was reduced to a minimum, the trunk showing a long, slow swelling which was not immediately reduced. The record now showed but little deviation from a straight line during the winter period, in which the variation in normal trees was at a minimum. (Fig. 12.)

The prevalence of conditions of temperature which induced growth in living trees late in January 1924 was followed by shrinkage in the trunk of this tree, which was noted as dead late in this month after an examination of the branches. The diameter of the base of the trunk decreased 0.9 mm. during the week beginning January 28, 1924, when shrinkage was checked by a heavy rain. The record now became a direct line, but with a daily reversible variation which was plainly discernible. (See fig. 13.)

Late in April, when the soil-moisture content began to run low, a daily equalizing variation of the dead stem amounting to 0.2 mm. was seen, it being the first time such a variation was observed in a trunk which was not alive. The shrinkage began about sunrise and continued until 4 to 6 p. m. When branches from the dead tree were placed with the bases in a solution of orange G the diffusion was irregular. In some cases the greatest extension of the dyed wood was in the same tract as that in which it occurred in the living stem, while in other cases it was near the central pith; the stained mass was irregular. The main stem was cut off near the base on May 24 and stepped into a vessel containing orange G solution. Two days later it was found that the dye had not gone far up the stem. The test was repeated with the same solution with 40 cm. of the basal portion cut away. The dye rose 18 cm. in 24 hours in the entire cross-section.

On May 21, 1924, a cavity which would hold 20 liters of water was dug near the base of the stem and filled with water several times, the liquid being quickly absorbed by the soil. No effects measurable by the dendrograph were observable on that day, but on the following day a slight and steady increase took place which amounted to 0.3 mm. three days later, at which time a slow contraction or shrinkage began.

If it be conceded that the daily reversible variations in the diameter of a trunk are to be taken as an indication of the existence of a continuous water column under tension in the wood cells, then the above observations constitute evidence of the movements of sap in dead stems without participation by any living cells whatever. No evidence of recent activity in any part of the tree was found when examined late in May. The mechanical disturbance of the system was limited to the removal of the leaves, which were plucked directly out of their sheaths, the scar being quickly sealed by the resinous excretion. The water column being intact, the narrow amplitude of the daily variation was due to the evaporation from surfaces in all parts of the tree which might be in connection with the water column in the wood cells. A thorough examination to determine whether or not any of these surfaces were of living cells was obviously incompatible with the continuation of the experiment. It was certain, however, that all cells underneath the bark and on the external surface of the trunk were dead and brown; it was also equally certain that no

leaves were present. It would seem, therefore, that a continuous water column subject to daily variations in tension was present in the tree. While, as Dixon has pointed out, a dead cell or an inanimate mass of colloids does not usually furnish the conditions for maintaining a water column in wood under tension, yet it seems to have done so in this instance.

Another experiment dealing with defoliation effects was made with Monterey pine No. 23. This tree had been stripped of its old leaves in June 1923. The leaves formed during the earlier part of the year were allowed to remain and, these having attained the greater part of their development, no noticeable alteration in the seasonal program of the tree could be seen. Enlargement of the trunk continued. Daily reversible variations after defoliation were marked and not noticeably different from those of control trees. Other experiments were performed with this tree, which would not affect the matter under discussion.[1]

The dendrographic record of the tree was continued throughout the winter of 1923–24 and through the growing-season which followed. Enlargement was still in progress late in August, although at a very low rate. The growing-season of 1923 terminated about the first of September. Enlargement during the week ending August 25, 1924, amounted to about 0.3 mm., and if undisturbed a similar accretion would doubtless have taken place in the following week. Growth began on February 26, 1924, and by the end of August an increase of over 5 mm. in diameter had taken place. The lowermost branches had made extensions of 10 to 25 cm. and the uppermost double this length during the season of 1924.

Complete defoliation was carried out on August 25, 26, and 27. 0.430 kg. of leaves were removed from the lower branches on the first day. Those matured in 1923 ran 16 to the gram, while the leaves of 1924 had not yet reached full size, their weight being but half that of the leaves of the previous year.

The leaves of 1923 from the median region of the tree were heavier, running 10 to the gram, while those formed during the present year on the same branches ran 23 to the gram. The total of the two classes from this region was 3 kg., the leaves from the terminal part of the tree weighing 3.110 kg., the ones formed in 1923 running 11 to the gram, and the ones developed during the season of 1924, 20 to the gram. The total number removed from the tree may be estimated as about 118,000, with a weight of 6.540 kg. The total transpiratory surface of the leaves removed may be taken to be about 1.5 sq. meter, using Peirce's estimate of the surface of a single needle to be 109 sq. mm.[2]

[1] MacDougal, D. T., and F. Shreve. Growth in trees and massive organs of plants. Carnegie Inst. Wash. Pub. 350. 1924. See pp. 25–27.

[2] Peirce, G. J. Notes on the Monterey pine. Botan. Gaz., 37, 448. 1904.

The sky was heavily overcast on the three days on which defoliation was carried out, and this would have tended to lessen the daily contraction slightly; it was, however, well-marked in control trees. The contraction was very slight on the second day. Beginning at sunset on the second day, an enlargement began which continued over the two succeeding days and for about 66 hours, which represented an increasing water-balance and a supposed lessened tension in the water column. A slight and gradual contraction for two days followed. After this, beginning August 31, on the sixth day after the beginning of defoliation was begun, the record included a daily slight but abrupt contraction followed by a slow recovery in a reversible series in which the diameter was not measurably altered. On foggy days the record was a direct line. The behavior described is in agreement with that of the tree defoliated in October, the slightly greater daily reversible variations in the tree described above being due to the greater transpiration in the summer season. (See fig. 13.)

The exudation pressures exhibited by the trunk, following defoliation, are of extraordinary interest. On August 28, the day after the removal of the terminal leaves, while the tree was in the middle of the 66-hour period of continuous enlargement, a pressure apparatus consisting of a short section of brass tubing screwed into a hole 1 cm. in diameter, 5 cm. deep, 30 cm. from the base, a short section of pressure tubing of rubber, and a closed end manometer were put in place. The readings shown in table 14 were made.

Table 14.

Date.	Time.	Length of air column in manometer.	Remarks.
		mm.	
Aug. 28	4 p. m.	65	
	4^h 30^m p. m.	67	"Negative" pressure of −2 mm.
Aug. 29	8 a. m.	45	
	9 a. m.	51	
	10 a. m.	51	
	11^h 30^m a. m.	52	Enlargement of trunk at an end.
	4 p. m.	52	Pressure, 1.25 atm.
Aug. 30	8 a. m.	54	Trunk stationary as to diameter for the previous 24 hours.
Aug. 31	8 a. m.	60	
	11^h 30^m a. m.	59	
	12 30 p. m.	59	
Sept. 1	8 a. m.	62	

The manometer was now dismounted and reset in a new hole a few centimeters higher up the trunk and in a sector 70° from the first attachment. The readings are given in table 15.

TABLE 15.

Date.	Time.	Length of column.	Remarks.
		mm.	
Sept. 1	8 a. m.	74	
	8^h 40^m a. m.	76	Absorption by tree; "negative" pressure.
	9 10 a. m.	77	
	9 30 a. m.	66	
	10 a. m.	66	
	11 a. m.	66	
	2 p. m.	59	
	5 p. m.	55	
	6^h 45^m p. m.	54	Maximum pressure = 1.3 + atm.
Sept. 2	8 a. m.	58	
	9 a. m.	56	
	11 a. m.	58	
	4^h 30^m p. m.	60	
Sept. 3	8 a. m.	62	

The manometer was taken down and fitted to an opening bored tangentially to the wood of 1922 in another sector and the readings given in table 16 obtained.

TABLE 16.

Date.	Time.	Length of column.	Remarks.
		mm.	
Sept. 3	2^h 45^m p. m.	76	
	2 55 p. m.	75	
	3 15 p. m.	71	
	3 30 p. m.	70	
	3 45 p. m.	68	
	4 15 p. m.	67	
	5 p. m.	64	
Sept. 4	7^h 30^m a. m.	51	
	9 a. m.	50	
	2 p. m.	48	Pressure 1.6 atm., a maximum which was maintained for about 20 hours.
	3^h 15^m p. m.	48	
Sept. 5	8 30 a. m.	50	
Sept. 7	11 a. m.	52	

The apparatus was now refitted to another opening. The readings are given in table 17.

TABLE 17.

Date.	Time.	Length of column.	Remarks.
		mm.	
Sept. 7	11 a. m.	76	
	11^h 10^m a. m.	77	Slight absorption by tree, giving "negative" pressure.
	4 30 a. m.	73	
Sept. 8	8 a. m.	68	Maximum pressure, 1.1 atm.
Sept. 9	8 a. m.	73	
	10 a. m.	75	
Sept. 10	10 a. m.	76	
Sept. 11	9 a. m.	77	
Sept. 12	2 p. m.	82	

The apparatus was now taken down and reset in the same manner in another sector several days later. Table 18 gives the readings.

TABLE 18.

Date.	Time.	Length of column.	Remarks.
		mm.	
Sept. 18	$2^h 30^m$ p. m....	68	
	3 p. m.........	69	
	$3^h 50^m$ p. m....	68	
Sept. 19	8 a. m.........	44	=1.5 atm.
	11 a. m.........	44	=1.5 atm.
	4 p. m.........	46	
Sept. 20	8 a. m.........	50	=1.36 atm.

Another test was made in mid-October with the results given in table 19.

TABLE 19.

Date.	Time.	Length of column of air in manometer.	Remarks.
		mm.	
Oct. 13	$11^h 20^m$ a. m....	68	
	11 30 a. m....	76	=0.9 atm. or 0.1 atm. "negative" pressure.
	12 noon........	68	
	2 p. m.........	65	
	4 p. m.........	61	
Oct. 14	8 a. m.........	56	
	12 noon.........	55	
	4 p. m.........	56	=1.2 atm.
Oct. 15	8 a. m.........	60	Exudation pressure decreasing.

The mercury column returned nearly to zero during the next two days.

The behavior of trees in which defoliation was carried out with a suite of young leaves of various stages of development in place afford some interesting comparisons. In such cases the movement of the water column and the tensions set up in it and the movement of electrolytes would be determined by the total transpiratory capacity of the young leaves, as well as the amount of photosynthetic product which may affect the welfare of the tree. The first experiment to be considered was one in which defoliation was carried out at the beginning of the growing-season, the leaves remaining being very young and small, and in close tufts at the ends of the branches.

A small tree, No. 27, comparable to the one described above, was fitted with a dendrograph in October 1923. No growth but the usual type of daily reversible variations are to be found in the record

until January 1924. No activity of the trunk or of the terminals occurred before mid-January when defoliation was carried out. Leaves were first stripped from the lower branches on January 15, the operation was carried forward on the 16th, and finished on the 17th. The leaves were heavier than in the autumn, 24 weighing a gram, and the total weighing 7.150 kg., from which it may be estimated that the tree was stripped of over 170,000 leaves, with an estimated surface area of about 1.8 sq. meters.

As controls and for the extension of the experiment, two smaller trees were defoliated at the same time. No. 27*a* stood a few meters away, was 1.1 meters high, with a stem 2 cm. in diameter 10 cm. from the base. This tree, which was estimated to have an age of about 7 years, bore leaves weighing 350 grams or a total of about 8,400 as the average weight was about the same as the larger tree. Defoliation of this tree was carried out on January 17. No. 27*b* was a larger tree, with a height of 2.6 meters and a diameter at the base of 29 mm. It probably was about 10 years old. 24 leaves weighed a gram and as the total had a draught of 800 grams the number may be estimated at about 19,000.

The dendrographic record of No. 27 shows that the daily reversible variation was of wide amplitude, as might be expected in a young tree during the winter months, and that this continued until defoliation was carried out on January 17. Beginning on the evening of the 16th, when two-thirds of the leaves had been removed and the transpiratory activity greatly reduced, the trunk began to swell, and the enlargement was continuous through the next day, when the process was completed. After this, the record was more nearly a direct line, the contraction being slight but abrupt and lasting only a few hours when the shrinkage was taken up.

Early in April, when normal trees were in a stage of most rapid growth, the dendrographic record of the defoliated tree showed a beginning progressive diminution of the stem which by mid-July had resulted in a loss of about 1.5 mm. in diameter. The reversible variations had become very gradual and irregular in pattern.

That such diminution in size following on stoppage of growth might rest on the deficiency of material, including sugars produced, lead to some sugar determinations of the contents of a stem of 27*b* and of normal or control stems. These determinations were made by Dr. Beverly L. Clarke by the method of Thomas and Dutcher.[1]

A portion of the entire stem, including the bark, was shaved and cut finely, so that no piece of the material showed a thickness of a millimeter. The determinations measured the amounts of active sugars which might be moving in the stems. In this case the elongated

[1] Thomas, W., and R. A. Dutcher. The colorimetric determination of carbohydrates in plants by the picric-acid reduction method. I: The estimation of reducing sugars and sucrose. Jour. Amer. Chem. Soc., 46, 1662–1675. July 1924.

node of the stem formed in 1922, which was half a meter in length, was taken. The normal specimen was much higher in water, having a moisture content of about 60 per cent, while that of the corresponding portion of the defoliated tree was much lower in water, having a moisture content of but 35.6 per cent. Based on the determinations of the reducing sugars present as dextrose, these amounted to 0.046 per cent of the fresh weight of the normal stem and 0.006 per cent of the defoliated stem. The proportion of reducing sugar to the dry weight of the normal stem was 0.011 per cent and in the defoliated stem 0.009 per cent. (See p. 27.)

It is notable that although the transpiratory apparatus had been reduced to such an extent as to minimize water-loss, yet the proportion of water in the stem was much below that of the control trees. Such a state of affairs could result only from the breaking of the column of water and the introduction of air-bubbles into the wood cells. That the continuous columns of water no longer existed in the wood cells was indicated by the fact that when this tree was cut close to the base, July 22, and stepped in dye, no upward conduction followed, the color penetrating the stem to only a few millimeters in two days and no differentiating action being shown.

No. 27, to which the dendrograph was attached, and which was much larger than 27*b*, survived for a longer period. The persistent shrinkage of the stem came to an end in mid-July. Daily reversible variations continued with diminishing range during the next two months, at which a serious examination was made of the entire tree. The daily variation during this period included an abrupt contraction in the morning hours which had reached its limit by noon. Enlargement began by 2 p. m., or even earlier on some days.

On September 17, 8 months after defoliation, the cambium was found to be in a collapsed condition and the ray cells were devoid of starch and seemed to be entirely devoid of life. Some of the branches were entirely dead and the tufts of undeveloped leaves borne by them were brownish. On other branches the leaves were beginning to flag and had an average weight of 90 to the gram. An almost complete ring of resin cells was seen in the cross-section of branches internal to 15 to 20 wood cells. The formation of this large number may be connected with the shock of defoliation. The wood of a branch taken at this time showed a water content of 35.5 per cent.

Wood of the two outer layers of a main branch which had three layers of wood was tested for reducing sugars by the method described above, with the result that the amount present was less than 0.03 per cent of the dry weight of the wood. This is one-third of the proportion present in the younger tree defoliated at the same time but tested a month earlier. It is evident that the carbohydrates in this tree were nearing exhaustion.

All of the cells external to the wood were dead, and cores taken from the stem with the increment borer showed that the outer layers of wood had acquired a yellowish tinge, in marked contrast with the whiteness of similar samples taken from normal trees. A hole bored into the outer layers was fitted with a metal tube filled with water and connecting with a manometer with a closed end. The air column, which was at first 63 mm. in length, was extended to 68 mm. by "negative pressure" due to the absorption of water by the wood. After a day this pressure was lessened slightly, but the mercury column did not return to zero and it was evident that no living or turgid cells capable of secretory action had been encountered.

A month later, October 16, 1924, a pressure apparatus was fitted to a bore 1.2 meter from the base. The length of the column of air in the closed end of the manometer, which was 95 mm., was not increased by negative pressure due to absorption, as was seen in so many instances. On the other hand, positive pressure compressed the air column to 92 mm. within an hour, after which no variations except those to be ascribed to temperature effects in the manometer were seen. After this test was closed, the manometer was fitted to another hole bored immediately below the heaviest whorl of branches nearly 2 meters from the base. The column of air in the closed end of the manometer was 94 mm. when the apparatus was adjusted at noon on October 20. Absorption of liquid from the tube began at once and by 3 p. m. the column of air had been lengthened to 98 mm. The length of the column was reduced to 96 mm. on the following day, but the slight negative pressure thus indicated was not lost. Fully three-fourths of the stem and branches of the tree were below the region tested, and in this part of the plant not enough living cells were present to set up exudation pressure.

A number of roots had been taken up in August and found to be entirely dead. The bark fell off in the hand and the wood was dry. The death of the cells had extended upward in the trunk at the time of the last measurements on root-pressure until no part of the stem or branches in any part of the tree, except possibly in the two internodes most recently formed, and these were not examined. Some of the wood cells contained a water column in September, and a few tufts of young leaves, green but in a wilted condition, were still in place on some of the upper branches. Their presence would constitute a series of living cells at the upper end of a cohesive column of water in a dead stem.

Inhibition or stoppage of growth may have been due to a lessened concentration of the carbohydrates, although, as may be seen from the above, a notable amount of reducing sugars was still present. Nothing is known as to possible deficiency of proteins or of accessory substances which might be necessary for growth. The stoppage of

movement in the water column would, of course, be accompanied by a cessation of upwardly moving electrolytes, which customarily diffuse laterally into the living cells.[1]

A semi-final examination of the tree was made January 16, 1925. The small tufts of undeveloped leaves were brittle and brown and, like the terminals which bore them, entirely dead. The bark came away in dry flakes, the phloem was brown and crumbling, and the wood yellowish brown. The remains of all cells were in place and it seemed possible that the colloidal material of the leaf-cells might furnish the evaporating menisci to sustain a water column in this tree, which was about 4 meters high. The daily reversible variations of the stem had an amplitude of 0.2 mm., or 1 part in 375, which would be about half that of a normal tree of this size. These variations are explainable on the basis of a cohesive column of water in the stem terminating in evaporating surfaces at the summit.

The next experiment in the series is one in which the young leaves had attained about half their full size at the time of removal of the older ones, and about half of the seasonal growth of the trunk had taken place. Half of the old leaves of Monterey pine No. 19 were removed on the afternoon of March 7, 1923, and the customary contraction of the stem did not take place on the following day. The remainder of the leaves were now removed, and no reversible variations were seen until the last week in May, nearly two months later, but again fairly definite by mid-June. A slight enlargement took place during a few days a month after defoliation, and a similar action began in mid-June, but at such a low rate that the total at the end of the season subsequent to defoliation was no more than 0.5 mm. in diameter.[2] This slight activity is in contrast with the behavior of Nos. 19, 25, and 27, which were defoliated in the resting season or at the beginning of the growing-season.

The young leaves of 1923, which attained about half the normal size in 1923, still bore that relation to the leaves of control trees. The average weight on September 16, 1924, ran about 30 to the gram in contrast with those of No. 23, which were nearly three times this weight.

The terminals developed in 1924 were not more than half the length of those of reduced length of the previous year. The extensions of the branches were from 5 to 10 cm. in length and the leaves developed on them were even smaller than the reduced leaves of the previous year. Their average weight was such that they ran 60 to the gram. Both determinations were made in mid-September 1924. The tree thus

[1] Some interesting data upon the "Effect of defoliation upon blossom bud formation" are presented by R. H. Roberts, in Research Bull. No. 56, Agric. Exper. Sta. of Wisconsin. Jan. 1923.

[2] MacDougal, D. T., and F. Shreve, Growth in trees and massive organs of plants. Carnegie Inst. Wash. Pub. 350, p. 24. 1924.

bore at the close of the active season of 1924 leaves of the previous year and of the present year, both of subnormal weight and size. It was estimated that at this time the photosynthetic and transpiratory apparatus had a capacity probably in correspondence with the reduced weight, or about a fourth that of a normal tree.

Enlargement of the trunk began on January 28, 1924, at about the same time as in control trees, and terminated May 9, at which time an increase of 1.4 mm. had taken place. This was probably about one-fourth of the normal activity of the tree, which made an enlargement of 5.3 mm. before March 8 in the season of 1924. This fact would be correlated with the reduction of the photosynthetic capacity of the lessened leaf-system, as noted above.

Reversible variations of a normal type prevailed at the beginning of the growing-season in 1924. Contraction was belated in the height of the growing-season, so that it did not begin until noon on many days and did not continue more than 3 or 4 hours. In March, the tree was in a state of expansion 18 hours of the day, although the amplitude of the daily change was as great as in a normal tree. After the close of the growing-season variation decreased in amplitude, so that by the end of July the weekly record did not depart widely from a direct line. This behavior continued even in the warm days of September, when conditions were highly favorable to transpiration.

The experiment made to close the cycle of the seasons was made with Monterey pine No. 29, which was defoliated May 28 to 31, 1924. This tree was 4 meters in height and 6 cm. in diameter at the base. A dendrograph had been attached on May 23. Actual enlargement of the stem was at an end for the season, and reversible variations were still marked.

On May 28, 700 c. c. of old leaves were removed from the upper part of the shoot, and 33 of these weighed 2 grams, so that the total was 10,550. The young leaves were about 4 cm. in length on the lower branches and about double this length on the terminal whorl.

On May 29, 1924, 920 grams of leaves were removed from the middle portion of the stem and branches, it being found that 40 of these weighed 2 grams, the total from this region being 18,400.

On May 30, 1924, the daily variation was as yet unaffected except that the pen rose slightly higher on the previous night, suggestive of reduced transpiration.

On May 31, the remainder of the leaves, weighing 1,550 grams, were removed in the morning; 44 of these weighed 2 grams, so that the total number of this lot was 34,000, and the total number for the entire tree was 63,000 with a surface area of nearly 1 sq. meter.

The leaves on the terminals of the current season had attained a greater size than those of No. 27; consequently they exerted a total osmotic pull greater than the much younger and smaller leaves which remained on No. 27 when it was defoliated in January.

In consequence of this, and probably also on account of the photosynthetic action in making material other than the carbohydrates, the reversible variations were of the usual type; some enlargement had also taken place after the operation. An increase, subsequent to defoliation, of 0.5 mm. in diameter had taken place by mid-July. Only the younger trees of similar size were growing during this period.

The effects of defoliation described here are much similar to those which were observed in No. 23, which have already been described. Defoliation on June 4, 1923, in a season in which growth of nearly all of the trees extended well past this date, showed reversible variations and some enlargement. The young leaves remaining on the shoots were larger than those on No. 29.

Growth of No. 23 began on January 27, 1924, and by mid-July an enlargement of 6 mm. in diameter had been made. The leaves were heavier than in June 1923. Samples weighed on July 22 showed 17 to the gram, while the weight at the time of the original operation was 30 to the gram (erroneously given as 90 in the publication cited). The terminals formed during the present year were hardly as long as those of 1923, but growth and extension in all trees in the drier year of 1924 were less than in the previous year.

The daily reversible variation in No. 29 showed a narrowing amplitude with the progress of the summer and had come down to a minimum by September. A sample of the wood of two outer layers of a branch of No. 29 taken September 18, 1924, showed a water-content of over 60 per cent, which is probably but little below normal for this season. A test for sugar by the picric-acid reducing method gave a content of 0.041 per cent. Some starch was seen in the ray cells. As the carbohydrate content is supposed to increase during the summer months, a determination of the contents of a normal stem was made. The moisture-content of a section of a branch of a normal tree taken September 21, 1924, used as a reference to the determinations in Nos. 27 and 29, was nearly 57 per cent of the whole. The reducing sugars of the two outer layers taken together amounted to 0.054 per cent of the dry weight. The earlier determinations showed a great disparity between the proportions of sugar in the outer and in the second layer and some further analyses of the two separately are described on page 77. At the same time, an increase of the amount present in normal trees with the progress of the season is seen.

A summarization of the effects of defoliation would include generalizations to the effect that a total loss of leaf-surface of the Monterey pine by removal of mature or nearly mature leaves in the autumnal or winter resting-period, even including the beginning of the growing-season, results in the death of the tree within a few months. The removal of the mature leaves soon after new leaves have started has such an effect. The operation in question deprives the tree of its

transpiratory surfaces. Some movement in the water-column is evidently indispensable to the Monterey pine at all times.

Defoliation also apparently prevents adequate photosynthesis, although much green is to be found in the young stems. A tree defoliated at the beginning of the growing-season carried on but little growth with its reserve food, and in late summer was found to have used up its reserves much more completely than any instance known to the writer, the sugar-content being extremely low and the starches almost completely exhausted.

Defoliation in the middle of the growing-season is carried out in such a manner that the newly formed leaves remain and the trees survive by reason of their activity. Such leaves fail to reach maturity, and trees which have come through the season with such restricted leaf action develop leaves in the following season which do not attain a weight more than one-fourth the normal. This would imply a lessened transpiratory activity and less movement in the water column, in addition to a sufficient supply of substances necessary for cell-formation.

A demonstration of the direct effect of lessened transpiratory action upon the movement of liquid in the stem was carried out as follows:

On May 31, 1924, two small pines about a meter in height were cut off at the base and stepped into small dishes containing a solution of fuchsin S 1: 1,000. A second pair was stepped into a solution of orange G of the same concentration. One of each pair was now stripped of its leaves. The results are given in table 20.

Table 20.

	Total absorption, 17 hours.	Length of colored stem.
	ml.	*cm.*
Fuchsin:		
Leafy shoot	40	37
Stripped shoot	35	29
Orange G:		
Leafy shoot	30	27
Stripped shoot	15	23

Both the rate and amount of colored solution passing into the stems was materially affected by the defoliation of the leaves. Some of the effects were visible within the first 2 hours.

Another pair of stems was prepared as above and stepped into solutions of erythrosin. One shoot was deprived of all except the leaves formed during the current year, which were now about two-thirds their full size. The results at the end of 24 hours are given in table 21.

TABLE 21.

	Absorption, 24 hrs.	Length of colored stem.	
		24 hrs.	48 hrs.
	ml.	*cm.*	*cm.*
Leafy shoot.........	25	....	....
Stripped shoot......	30	70	30

A day later the following anomalous results were shown in 6 hours:

Leafy shoot, 30 ml., and a conduction to 25 cm.
Stripped shoot, 15 ml., and a conduction to 20 cm.

These tests were repeated with two older trees. Two pine trees about 2 meters high were cut and the bases stepped in erythrosin at 11 a. m. June 2, 1924. One, which proved to have 11 annual layers with a thickness at base of 2 cm., was defoliated. In 23 hours it took up 65 ml. of the solution and conducted the dye about 63 cm. The other, with identical basal diameter and with only 8 layers, took up 55 ml. of the dye and conducted the color to about the same distance in the stem, the second layer in both cases showing the greatest conduction. The stems were shortened by cutting away the stained parts and again stepped in the erythrosin solution. 24 hours later, the remaining part of the defoliated shoot had conducted the dye 40 cm. from the base and used 40 ml. of the solution. The leafy stem had conducted the dye 62 mm. and used 80 ml. The total action of the two stems was as follows:

Leafy shoot, conduction 125 cm.; absorption 145 ml.
Defoliated, conduction 103 cm.; absorption 105 ml.

The results of these tests make it evident that the removal of the older leaves of a shoot lessens the pull on the stem by which solution is taken at its cut base. Dyes in lesser quantity are taken and are conducted shorter distances than in leafy shoots. It is to be noted that these effects are in stems taken at a season when defoliation does not modify the action of the stem to such an extent as to change the dendrographic record in any important feature. It remains to be seen what effects might be secured by the repetition of the experiments in the height of the growing-season.

The removal of the leaves of the previous year, about the end of the growing-season for the trunk, has a minimum effect in comparison with defoliation at other times or with complete defoliation. The operation at this time is at the beginning of a period when the major use of sugars in wood formation is at an end and the total daily transpiratory loss from the tree is progressively diminishing. The suite of leaves of the current year, although not yet of full size and weight,

are capable of an amount of activity which results in a procedure nearly normal in the summer and autumnal resting-periods. The amount of sugar present in the wood at the end of the summer, however, is something less than in a normal tree.

It is notable that the defoliation tests show that the Monterey pine is a tree to which complete defoliation is a fatal injury. The removal of all mature leaves at any time during the resting-period, or even when the new leaves have made some little development, results in death. The activity of a suite of leaves which have reached a stage of at least half of their full development is necessary for the life of the tree. An accumulation of surplus carbohydrates, which is so notable a feature of many trees, is reduced to its lowest terms in the Monterey pine. At the same time, it was found that when a tree is defoliated the tree may make use of its accumulated material much more completely than is ordinarily seen in plants. Nearly all of the starch disappears and the sugars are reduced to a very low proportion in the stem.

GENERAL SUMMARY.

The results described in the foregoing paper unanimously support the conception of the cohesive column of water of Dixon as the main feature of the hydrostatics and movement of liquids in trees.

The daily reversible variations in the diameter of trunks, which were first announced by the author to be connected with the changes in the thickness of wood-cells with varying tension on the liquid and gaseous contents, have been studied in detail in the Monterey pine. The reduction of vapor-pressure externally to the transpiring cells of the leaves consequent upon the opening of the stomata, and the action of light and rising temperature in the morning lowers the vapor-pressure of the water in the cell-walls, causing evaporation and the retreat of the menisci of the water into the interstices of the wall. The resultant increased pull exerted on the top of the complex water column, which depends from these surfaces and extends to the roots, increases the tension in the contents of the wood-cells, which contract in diameter. This effect is so direct that increased evaporation from the leaves of a tall tree is followed by the contraction of the base of the trunk in a few minutes. The contraction takes place chiefly in the conducting layers, usually the second, third, and fourth of the Monterey pine. It has not been possible to calculate the degree of shrinkage in these layers, but the change may amount to as much as 1 part in 170 in the entire trunk of small trees and as little as 1 part in 1,250 in older ones. The actual contraction in the layers serving as conduits may be as much as 2 or 3 per cent of their diameter.

If stems are cut and quickly stepped into solutions of dye, the path of the color up the stem may be taken to represent the location of the complex water column under tension which extends to the roots from the leaves. It is to be noted, however, as the stain ascends in the layers usually followed at a rate from 2 to 10 cm. per hour, that such dyes as acid fuchsin also pass radially along the ray tracheids, so that at the end of such an experiment the color will be found in other layers external and internal to the ones in which the main upward movement takes place. The complex water column of the Monterey pine occupies the layers directly connected with the leaves, which include the second, third, and sometimes the fourth from the outside in the parts of the tree more than 2 years old. When only two layers are present as the terminal parts, both generally stain quickly.

Electrolytes enter the plant through the living parts of the root at a rate and in a proportion determined by their own ionic mobilities and as modified by their interaction with the colloidal materials in the endodermal membrane, which is taken in this meaning to include all of

the layers of living cells between the epidermis and the xylem. Water is pulled through the same layer by osmotic action. Some organic matter from maturing cells passes into the xylem, with the result that the upwardly moving stream carries both salts and organic compounds. Perforations in the membranes of the pits in the wood may afford a continuous passage for all particles carried.

No direct measurements of the pulling power of transpiration were made, or of the resistances presented by the Monterey pine. It was found, however, that when the stump made by excising the terminal of a small tree was connected with an air-pump a noticeable increase in conduction of liquids resulted, in lengths of 2 or 3 meters, the air-pump replacing leafy branches and probably increasing the total pull on the water column not more than half an atmosphere. Basally applied pressures, using a column of mercury, also resulted in accelerations with some modifications of the path of solutions forced into the bases of stems.

The secretory action by which living cells take in water osmotically and force it through walls common with non-living elements is considered without reference to pressures set up in roots. Such an action doubtless does take place in roots and in small plants; the water thus forced into the xylem may be carried up the entire length of the stem to the leaves. This action would be most noticeable and marked in stems in which tracheæ and elongated conduits were present. The Coniferæ have been hitherto characterized as trees in which such "root-pressures" were lacking or present in small degree.

The living ray cells are to be considered as capable of exerting such exudation pressure. When manometers are coupled to the stump of a pine stem by a pressure-tube containing water, direct connection is made with a large interior cross-section of the stem with a possible open space in the region of the pith, and with air-filled wood, so that the exudation pressure exerted by the living cells is taken up as fast as it is developed, with the result that minimum or negative readings are obtained.

A new method has been developed for the measurement of exudation pressures in trunks, and the Monterey pine offers material exceptionally advantageous for testing this matter. Vigorously growing trees lay down as much as 8 or 10 mm. of wood per year, and the complex column of water occupies three or four of these layers, in which presumably the ray parenchyma is also alive. A hole which is bored tangentially into a stem, terminating in these layers, taps a large amount of tissue in which living cells form a large proportion, and these pour their exudates into the cavity. A metal tube fitted to this cavity by screwing in one threaded end and connected with a manometer may be expected to register the varying pressure set up in the cavity. Some absorption of water at the beginning of the test gave negative

readings after which positive pressures increased for a period of about 18 to 48 hours, following which a decrease ensued. The maximum reading is a result of the mechanical action of the manometer and of the exosmotic or excretory action of the living cells. The manometer registered the maxima of 3.5 atmospheres of pressure only after a distinct amount of liquid has been excreted, which in the case noted amounted to 0.25 c. c. Theoretically the maximum osmotic action would be shown when the sap was most concentrated, which is at the exact beginning of the experiment. Perfection of the method described above would doubtless increase the maxima described in this paper. Measurements of this kind in *Betula*, in which the volume of the sap excreted is very great, would probably give some high values. It was not possible to establish any periodicity in the action outside of the general course noted above. Some temperature effects were to be attributed directly to the instrument. No correlation with the transpiratory action of the leaves or with the reversible variations of the trunk could be found.

The sealing action of resinous exudations in the pine is such that exudation is soon stopped. The higher maxima described by Figdor, Boehm, and Molisch were obtained from trees which showed long continued exudations from stumps or cavities in the trunks.

Similar methods were used in measuring exudation pressures in the large thickened roots of pines. Metal tubes were screwed into cavities bored into the tissues. Preliminary "negative" pressures were observed after which positive pressures from 1.5 to 2 atmospheres were developed within 40 to 48 hours. Here, as in the trunks, no periodicities could be made out. Neither could any correlation with the diurnal expansion and contraction of the root be established.

An anatomical examination of the stem shows elongated conduits in the protoxylem only, where as many as 440 spiral ducts were found near the pith. These and spaces left by the collapse of the pith make a passage through the stem which makes any measurement of exudation pressures by tubes fastened to stumps of but little value. The medullary rays include tracheids and parenchymal cells with heavy walls which soon become empty and lignified. The thin-walled cells of the rays, which are loaded with starch and carbohydrates, participate in the osmotic action which may set up exudation pressure. These active cells occupy the middle portion of the rays, comprising 85 per cent of its bulk, the tracheids and thickened parenchyma cells being above and below. The pits in the walls of these cells may play a part in the secretory action of such cells, as exosmose would probably take place through the thin membrane. Another source of action which might result in exudation pressure would be the parenchyma cells surrounding the resin ducts, which have a length of as much as 140 mm. The secretory action of such cells would force liquid into the resin

canals which would be expressed as exudation pressure when the stem is tapped. It is also to be noted that resin may exert some pressure following imbibition.

In view of the high exudation pressures observed in the Monterey pine, much higher than have been previously recorded for any plant, it is remarkable that the amount of reserve material in the trunk is comparatively small. The medullary rays are low and carry starch only in the parenchymatous cells with thin walls. The depletion of this material follows within a comparatively short time, when, photosynthesis being canceled by defoliation, the stored carbohydrates constitute the only available food-supply.

Two methods of experimentation, girdling of trunks and topping and defoliation, deserve separate discussion, since the entailed operations work alterations in several major processes in the tree. The removal of the bark and all soft cells external to the wood of a Monterey pine is followed by the blocking of the tracheids of the outermost layer of wood, which cancels this tract as a conductor of liquids either up or down the trunk. During the summer season this layer may contain 30 times as much sugar as the layer internal to it. Girdling, however carefully done, would therefore block the passage of sugars in either direction in this layer. There is much evidence to support the suggestion that the downward movement of organic material may take place partly in this layer. The matter, however, needs detailed experimental tests. In the pine, as in other trees, girdling is followed by the accumulaton of carbohydrate above the exposed zone and growth is greatest in this region.

Girdling with boiling oil, which heats and kills the tree to the center, results in the breaking of the water column and the death of the tree.

Girdling of a rapidly growing tree may cause only temporary effects in some cases. These temporary effects include diminished daily reversible variations in the diameter of the stem.

Removing the leafy branches and terminals of a pine tree not only disturbs the continuous water column, but eliminates the photosynthetic apparatus. When this is done completely death results within a short time. A topped tree with a few branches may survive for a year or two. One with many remaining branches may go on indefinitely.

Defoliation of the pine may be done without breaking the water column in the trunk, and this operation has been carried out on trees at various seasons. The removal of all green leaves from a Monterey pine in the resting-period or in winter results in its death within a few months. The removal of all mature leaves during the growing-season, when the young leaves are only partly formed, stops growth and lessens daily reversible variations for a period of at least two years. The removal of leaves of previous years, allowing the most recent ones

to remain, the operation being performed at the end of the growing-season of the trunk, causes a minimum disturbance. Defoliation is invariably followed by a lessened carbohydrate of the stem.

The scars resulting from the removal of the leaves are quickly sealed with resinous material, so that the water column remains intact; consequently, the trunk swells for a period of 2 or 3 days after the operation. The subsequent daily reversible variations are minimized. The colloidal remains of the evaporating cells in the dead leaves and terminals would furnish evaporating menisci, the action of which would sustain a cohesive column of water. It seems clear that such variations persist in trees which are dead from the tips of the roots. Such trees show a diminished water-content of the trunk and a very small amount of sugar in the wood cells. No exudation pressures are possible under such conditions, but a manometer connected with a hole bored into the trunk shows slight negative pressures, due to the absorption of water from the tubes of the instrument.